Toxicity of Plastic Consumer Products:

A Biological, Chemical and Social-Ecological Analysis

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"Plastic is forever …

and a lot cheaper than diamonds."

advertising slogan 1980s

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Abstract

Plastics contain a complex mixture of chemicals including polymers, additives, starting substances and side-products of processing. These plastic chemicals are prone to leach into the packaged goods, in the case of food contact materials (FCMs), or into the natural environment, in the case of plastic debris. Thus, plastics represent an exposure source of chemicals for humans and wildlife alike. While it is widely known that individual plastic chemicals, such as bisphenol A and phthalates, are hazardous, little is known on the overall chemical composition and toxicity of plastics. When fragmented into smaller particles, referred to as microplastics (< 5 mm), the plastic itself can be ingested by many species. It is well established that microplastic ingestion can have negative consequences for a wide range of organisms including invertebrates, but the contribution of plastic chemicals to the toxicity of microplastics is unclear.

Given the above, the present thesis aimed at a comprehensive toxicological, ecotoxicological and chemical characterization of everyday plastics. For a comparative evaluation, 77 plastic products were selected covering 16 material types (e.g., polyethylene) made from petroleum or renewable feedstocks. These products included biodegradable products, FCMs and non-FCMs, as well as raw materials and final products, respectively. In the first two studies, the chemical mixtures contained in the 77 products were extracted with methanol and extracts were analyzed in a set of four *in vitro* bioassays and by non-target high-resolution gas or liquid chromatography mass spectrometry. Since an exposure only occurs if chemicals actually leach under realistic conditions, in a third study migration experiments with water were conducted for 24 out of the 77 products. The aqueous migrates were assessed in the same way as the methanolic extracts. In addition, the freshwater invertebrate *Daphnia magna* was exposed chronically to microplastics made of polyvinylchloride (PVC), polyurethane (PUR) and polylactic acid (PLA) to investigate the contribution of chemicals in microplastic toxicity, in a fourth study.

The experimental findings demonstrate that a wide variety of chemicals is present in plastics. A single plastic product can contain up to several thousand chemical features, most of which unique to that product and at the same time unknown. The results also indicate that the majority of these chemical mixtures are toxic *in vitro*. Accordingly, 65% of the plastic extracts induced baseline toxicity and 42% an oxidative stress response, while 25% had an antiandrogenic and 6% an estrogenic activity. This implies that chemicals causing unspecific

toxicity are more prevalent in plastics than such with endocrine effects. These chemicals can also leach from plastics under realistic conditions. Between 17 and 8936 chemical features were detected in a single migrate sample and all 24 tested migrates induced *in vitro* toxicity. This means that humans and wildlife can actually be exposed to toxic plastic chemicals under realistic conditions. Generally, each product has its individual toxicological and chemical fingerprint. Thus, neither material type, feedstock, biodegradability nor the food contact suitability of a product can serve as a predictor for the toxicity, the chemical composition or complexity of a product. Likewise, this means that bio-based and biodegradable materials are not superior to their petroleum-based counterparts from a toxicological perspective despite being promoted as sustainable alternatives to conventional plastics.

Moreover, the present thesis demonstrates that plastic chemicals can be the main driver for microplastic toxicity. Irregular microplastics made of PVC, PUR and PLA adversely affected life-history traits of *D. magna* in a polymer type- and endpoint-dependent manner at concentrations between 100 and 500 mg L^{-1} and with a higher efficiency than natural kaolin particles. While the toxicity of PVC was triggered by the chemicals used in the material, the effects of PUR and PLA were induced by the physical properties of the particle.

In addition, in the fifth study, results and observations made during this thesis were integrated inter- and transdisciplinarily with the perspectives of a social scientist and a product manufacturer. This elucidated that knowledge on plastic ingredients is often concealed, is lacking or not applicable in practice. These intransparencies hinder the safety evaluation of plastic products as well as the choice and sale of the least toxic packaging material.

Overall, the present thesis highlights that the chemical safety of plastics and their bio-based and biodegradable alternatives is currently not ensured. Thus, chemicals require more consideration in the toxicity and risk assessment of plastics and microplastics. Product-specific and complex chemical compositions, including unknown compounds, pose a challenge here. Two essential steps towards non-toxic products are to increase transparency along the product life cycle and to reduce the chemical complexity of plastics by communication and regulation. The results of the present thesis indicate that products exist which do not contain toxic chemicals. These can serve to direct the design of safer plastics. Since toxicity and chemical complexity seem to increase with processing, the integration of toxicity testing during the production steps would further support the safe and sustainable production and use of plastic products.

Abbreviations

BPA	Bisphenol A
Bio-PE	Bio-based polyethylene
Bio-PET	Bio-based polyethylene terephthalate
CCS	Chemicals strategy for sustainability
EC	European Commission
ECHA	European Chemicals Agency
ECx	x% effect concentration
$EC_{50Repro}$	Concentration reducing the reproduction by 50%
EDA	Effect-directed analysis
EDC	Endocrine disrupting chemical
EPS	Expanded polystyrene
EU	European Union
EPR	Extended producer responsibility
FCM	Food contact material
GC	Gas chromatography
HDPE	High-density polyethylene
IAS	Intentionally added substances
LC	Liquid chromatography
LDPE	Low-density polyethylene
LOD	Limit of detection
MEC	Measured environmental concentration
MDPE	Medium-density polyethylene
MS	Mass spectrometry
NIAS	Non-intentionally added substances
OML	Overall migration limit
PBAT	Polybutylene adipate terephthalate
PBS	Polybutylene succinate
PE	Polyethylene
PEC	Predicted environmental concentration
PET	Polyethylene terephthalate
PHA	Polyhydroxyalkanoate

PLA	Polylactic acid
PNEC	Predicted no-effect concentration
PP	Polypropylene
PS	Polystyrene
PUR	Polyurethane
PVC	Polyvinyl chloride
QTOF	Quadrupole time-of-flight
REACH	Regulation (EC) 1907/2006 concerning the registration, evaluation, authorization, and restriction of chemicals
ROS	Reactive oxygen species
SAPEA	Science Advice for Policy by European Academies
SML	Specific migration limit
SPE	Solid phase extraction
SSD	Species sensitivity distribution
SVHC	Substance of very high concern
UPLC	Ultra-high performance liquid chromatography

1 Introduction

1.1 Rise of the plastic age

Plastics have become an integral and indispensable part of everyday life. They brought technical innovations (e.g., light-weight construction has made commercial air travel possible) and societal benefits (e.g., sterile packaging has improved public health). The first synthetic plastic, Bakelite, was invented in the early 20th century, but only in the last 70 years has global plastic production increased exponentially to 359 million tonnes[1] in 2018 (Geyer et al., 2017; PlasticsEurope, 2019). Besides being light-weight, plastics are unbreakable, malleable and have good thermal as well as barrier properties (Andrady and Neal, 2009). Due to these favorable characteristics, their versatile applicability and low-cost, they outperform and have substituted alternative materials such as glass, paper, metal and ceramics in many sectors. Packaging represents the major application field of plastics with a share of 39.9% in the European plastic demand, followed by the building and construction industries (19.8%), in addition to the automotive industry (9.9%; PlasticsEurope, 2019).

1.2 Plastics in use

Plastics are a chemically complex and diverse set of materials, where branched and interacting polymer chains build their ground-structure. Here, the polymer types polypropylene (PP, 19.3%), polyethylene (PE, 29.7%), polyvinyl chloride (PVC, 10.0%), polyurethane (PUR, 7.9%), polyethylene terephthalate (PET, 7.7%), as well as polystyrene (PS) and expanded PS (EPS, 6.4%), have the highest market share (PlasticsEurope, 2019).[2] Blending with additives, such as plasticizers, stabilizers, antioxidants, flame retardants and colorants, in addition to fillers, facilitates processing or improves the material's physical, chemical or electrical functionality. Further chemicals contained in the final product include residual monomers and oligomers, as well as non-intentionally added substances (NIAS), such as impurities and side- and breakdown products of polymerization and compounding

[1] Without plastic fibers.
[2] Demand in Europe 2018.

(Muncke, 2009). Since most of these plastic chemicals[3] are not covalently bound to the polymer matrix, they can transfer to the air via volatilization as well as to the packaged goods via migration. Furthermore, they can enter terrestrial and aquatic environments. In this way, they become available for human and wildlife exposure (Andrady, 2011). Plastic chemicals have been measured in virtually every human and wildlife tested in the past decade (Bergman et al., 2013; Bushnik et al., 2010). Until now, the exposure and hazards of only a few prominent compounds, e.g., bisphenol A (BPA) and phthalates, have been assessed extensively (Groh et al., 2019; Wagner, 2017) and addressed by specific regulations. The European Union (EU) regulation 321/2011, for instance, restricts the use of BPA in infant feeding bottles made of polycarbonate (EU, 2011b). However, more than 4700 intentionally added substances (IAS) have been associated with plastic food contact materials (FCMs; Groh et al., 2020) and more than 4200 chemicals, including NIAS, with plastic packaging alone (Groh et al., 2019). Of the former, 325 were classified as hazardous.

In the regulatory context, chemicals used in plastic manufacturing generally have to conform with REACH, the European regulation concerning the registration, evaluation, authorization and restriction of chemicals (EU, 2006). However, REACH excludes polymers since, due to their high molecular weight, they are considered of low concern. FCMs including plastics are regulated separately (European Commission (EC) No 1935/2004; EU, 2004); this means that a chemical identified as a substance of very high concern (SVHC) under REACH may still be used in FCMs (Geueke and Muncke, 2018). Plastic FCMs and articles, additionally, have to conform with regulation (EC) No 10/2011 that addresses mono- and multilayer products as well as coatings (EU, 2011a). It includes a positive list of monomers and other starting substances, additives and polymer production aids that may be used in plastic FCMs but not transfer to foodstuff by more than their specific migration limits (SMLs) or more than 60 mg kg^{-1} foodstuff. As a whole, plastic materials should not transfer their constituents to food simulants exceeding the overall migration limit (OML) of 10 mg dm^{-2} food contact surface. One deficiency attributed to this regulation is that these limits are based on outdated and publicly inaccessible hazard and exposure estimates. Furthermore, the regulation only prescribes the chemical analysis of selected target compounds while disregarding colorants, solvents, NIAS and unknown compounds present in the end product, as well as mixture toxicities (Muncke et al., 2017). Due to these and further reasons, critique has been raised over the adequacy of the approach to determine chemical

[3]The term ‘plastic chemicals’ includes all intentionally and non-intentionally added substances present in plastics.

exposure from and risk of FCMs (Groh and Muncke, 2017). Concerns are strengthened by *in vitro* and *in vivo* studies indicating that chemical mixtures of plastic FCMs can be toxic (e.g., Severin et al., 2017; Wagner and Oehlmann, 2009). Proposed improvements to warrant the safety of plastic FCMs include assessing the mixture toxicities of final products by a battery of *in vitro* bioassays and identifying the compounds of concern by chemical analysis (Groh and Muncke, 2017). Simultaneously, this approach would help to further clarify the chemical diversity and overall toxicity of plastics.

While knowledge on the safety of plastic chemicals is still fragmented, the downsides of plastics related to environmental costs are more obvious. Accordingly, the production of conventional plastics demands a small but significant fraction of fossil fuels as feedstock[4] and processing is connected with externalities such as carbon dioxide (CO_2) emission. Furthermore, plastics are often single-use and have a short usage life, as is the case for the majority of packaging (Andrady et al., 2015). After disposal, 32.5% of collected European plastic waste is recycled chemically or mechanically,[5] 42.6% is used for energy recovery and 24.9% ends up in landfills (PlasticsEurope, 2019). From landfills and other inadequate post-consumer waste management, plastics spill into the environment. In addition, only 29.1 million of the 61.8 million metric tons of plastics produced in Europe in 2018 were collected as waste at all and, thus, a high proportion is lost (e.g., due to improper disposal or loss during use).[6] This raises questions about the fate of the latter. In 2016 alone, 19–23 million metric tons of plastics entered aquatic ecosystems (Borrelle et al., 2020). Here, they accumulate due to the persistency of the material. Plastics are now so ubiquitous in the environment that they have been proposed to be a geological indicator for the start of the Anthropocene – or the 'plastic age' (Brandon et al., 2019; Geyer et al., 2017).

Bioplastics (e.g., bio-based polyethylene, Bio-PE, and polylactic acid, PLA) as well as plant-based blends (e.g., made of starch, cellulose or bamboo) are marketed as a sustainable alternative to petroleum-based plastics since they are either made from renewable resources (bio-based), are supposed to degrade naturally (biodegradable) or both. However, they do not necessarily outperform conventional plastics in all aspects. For instance, they can have an overall similar environmental impact (Gironi and Piemonte, 2011a) and natural degradation only takes place under certain conditions (Haider et al., 2019). Furthermore, particularly little

[4] 4–6% of total fossil-carbon resources are used to produce plastics (Chiellini et al., 2018).

[5] Mechanical recycling means the reprocessing into a product with the same (closed-loop recycling) or lower (downgrading) properties while chemical recycling is the de-polymerization into chemical components, also referred to as feedstock recycling (Hopewell et al., 2009).

[6] It has to be considered that not every plastic becomes waste the same year it is produced and that plastic demand is continuously increasing (PlasticsEurope, 2019).

is known with regard to the chemicals they contain and the safety of these compounds (Scarfato et al., 2015); this is especially problematic because, as to their name suggests, bioplastics may be prone to end up in natural environments (Zhu and Wang, 2020).

1.3 Plastics in the environment

When plastics end up as litter in the environment, the material's advantageous properties, such as persistency and low weight, turn into disadvantages. The light-weight of plastics facilitates wind- and water-driven transportation which results in an ubiquitous distribution across terrestrial and aquatic ecosystems (Jambeck et al., 2015; Souza Machado et al., 2018). In the aquatic environment, plastics can harm species through entanglement and unintentional capture from ghost nets (Ivar do Sul and Costa, 2014). Biological, chemical and physical conditions and processes, such as UV-light, oxygen, temperature and mechanical abrasion, cause continuous degradation and fragmentation of the persistent material (Gewert et al., 2015). The resulting particles are commonly referred to as microplastics upon reaching sizes of < 5 mm (Arthur et al., 2009).[7] Apart from ageing mechanisms in the environment, microplastics can also be generated from abrasion during use, as is the case for tire wear. In contrast to theses secondary microplastics that originate from larger plastics, primary microplastics are intentionally produced in small sizes, as is the case for microbeads in facial-cleansers (Cole et al., 2011).

First documented in 1972 (Carpenter and Smith, 1972) and termed 'microplastics' in 2004 (Thompson et al., 2004), nowadays their presence is reported in virtually every environmental compartment from marine and freshwaters to deep sea sediments and the arctic snow (Bergmann et al., 2019; Courtene-Jones et al., 2017). Hereby, the measured concentrations cover a wide range depending on the location, the sampling method and the type of analysis applied. Consequently, global microplastic concentrations in oceans range from 0.014 to 12.5 particles m^{-3} in the water phase and from 185 to 80,000 particles m^{-3} in sediments. Corresponding concentrations in rivers are 0.17 to 3.45×10^5 particles m^{-3} and 5.15×10^2 to 6.49×10^7 particles m^{-3}, respectively (Hidalgo-Ruz et al., 2012; Scherer et al., 2020). However, lacks of harmonization and methodological limitations[8] complicate the

[7] It is to be noted that a scientific consensus on the exact size definition of 'micro'plastics has not yet been achieved (Hartmann et al., 2019).

[8] For instance, the smallest single particle that has yet been detected with analytical methods was 1 µm but most current analysis methods can only detected particles > 10 µm (Anger et al., 2018)

comparison of microplastic concentrations between studies and prevent realistic abundance estimates, respectively.

The wide size spectrum of plastics allows an uptake by species of all tropic levels, feeding types and habitats, including seabirds, fish, zooplankton and phytoplankton (Franzellitti et al., 2019; Wang et al., 2020). Once taken up, with their particle properties, microplastics can cause external and internal physical damage, such as injuries of the digestive tract, as well as reduced food resorption resulting in nutritional deficiencies (Ruijter et al., 2020). As a consequence, changes occur in energy reserves, growth, reproduction and mortality rates. Upon tissue translocation, the micro-sized particles can also bring about inflammation and necrosis (Kögel et al., 2019; Ramsperger et al., 2020; Wright et al., 2013). Just as plastic products in use, microplastics are characterized by a complex chemical composition. These inherent chemicals can leach from microplastics and the higher surface-to-volume ratio of microplastics compared to larger plastics promotes chemical release (Barnes et al., 2009). Exposure of aquatic animals to these plastic chemicals has been associated with several adverse effects (e.g., Oliviero et al., 2019; Seuront et al., 2021). In addition to the chemicals inherently present in the material, due to their physicochemical properties such as hydrophobicity, microplastics can further absorb hydrophobic organic pollutants from environmental compartments and transfer them into organisms. Since sorption and desorption is highly context-dependent, microplastics may increase or decrease animal exposure to contaminants (Koelmans et al., 2016).[9] To date, research on the chemical component of microplastic toxicity has prioritized compounds acquired from the environment, although it has been suggested that additives used in plastics are more toxic (Browne et al., 2013; Nobre et al., 2015). Along this line, some plastic chemicals, such as endocrine disrupting chemicals (EDCs), may not follow the premise of toxicology that 'the dose makes the poison'[10] but cause adverse effects at low concentrations while no or other effects at high concentrations (Vandenberg, 2014).

The composition of chemicals, inherent and sorbed, is manifold as are physical characteristics of microplastics that comprise different particle shapes, sizes, and surface morphologies. On the other hand, microplastic properties are ever-changing via aggregation and fragmentation, as well as the association and dissociation of chemicals and biota (Alimi et

[9]Sorption and desorption are ongoing until a chemical equilibrium has been reached between compartments. This depends on the concentration of the chemical in the different compartments, the properties of the chemical, the microplastic and the surrounding medium (Koelmans et al., 2016).

[10]The original quote is: "*All things are poison, and nothing is without poison; only the dose permits something not to be poisonous*" (Paracelsus, 1589).

al., 2018; Rummel et al., 2017). This outlines the notion that microplastics are not one homogeneous entity but a group of contaminants (Rochman et al., 2019). At the start of this thesis, ecotoxicological studies with microplastics rarely reflected this substantial complexity and diversity that is present in the environment. Along this line, mostly spherical microplastics based on PS were investigated even though irregular fragments made of PE are predominantly detected in the environment (Burns and Boxall, 2018). Furthermore, exposures with naturally occurring particles were missing, although they represent an essential reference to investigate whether observed toxicities are microplastic-specific and not particle-related. Moreover, the chemicals contained in (micro)plastics have mostly been disregarded in analyses and effect reporting. Consequently, the prevailing question at the start of this thesis was not whether microplastics cause adverse effects to organisms, but, which component (e.g., physical or chemical characteristic) is responsible for it.

Beyond that, impacts are not limited to single organisms or generations but microplastics can induce multigenerational effects (Schür et al., 2020) and may change whole habitats. For instance, plastic litter can transport and disseminate species (‘plastisphere’) including pathogens (Barnes and Milner, 2005; Kirstein et al., 2016) and can alter sediment properties (Carson et al., 2011). This shows that microplastics in natural environments are not only a diverse, complex and dynamic material group, but also act on several scales. These aspects complicate the evaluation and integration of all impacts associated with microplastics and, thus, the assessment of their overall risks. In this context, the applicability of classical environmental risk assessment of chemicals to phenomena such as microplastics is critically discussed (SAPEA, 2019). Here, the predicted no-effect-concentration (PNEC; above which an effect is likely) is compared to the measured (MEC) or predicted environmental concentration (PEC). If PEC or MEC > PNEC, a risk for the environment is potentially present (EC, 2003). Hence, it is calculated under which circumstances certain harm is likely. This helps to identify causes and to predict outcomes of chemical exposure.

1.4 Plastics as a systemic risk

Conventionally applied risk assessment procedures, such as the described above, are based on the assumption of a monotonic linkage between a well-identified hazard source and endpoint (OECD, 2003). However, pollution with plastics and microplastics represents a wicked problem. Accordingly, plastics are difficult to define (e.g., due to their material complexity), are unstable (e.g., ageing changes the material properties, the amount of plastic litter is

increasing), disperse over various spatial scales (e.g., air, land, water, across borders) and unfold over long temporal scales (e.g., due to their persistence) as well as are having potential global implications (e.g., dislocation of species attached to plastics). Furthermore, different disciplines are needed to understand this wicked phenomenon, for instance, natural sciences that assess the impacts of plastic pollution and social sciences that evaluate the benefits of plastics. Since benefits (e.g., lightweight, unbreakable and sterile packaging) and downsides of plastics (e.g., persistent waste) are closely intertwined, plastics pose a complex social-ecological problem. Due to the complex interdependencies, efforts to solve one aspect of the problem may create another problem; for instance, changing from plastics to glass decreases plastic consumption but increases the ecological footprint due to energy-intensive transportation of heavy glass (Burke et al., 2017; NRC, 2012).

This outlined 'wickedness' is a central aspect towards framing plastics in the environment as systemic risk.[11] Another aspect of the plastic phenomenon that conforms to systems risk is the decentralized origin of plastic pollution: It is as a side-product of everyday life instead of being caused by one single accident or natural disaster. In addition, the source and the target of the problem are decoupled spatially (e.g., location of litter production and occurrence differ) and system-wise (e.g., plastic generates profits in trade but leads to economic loss when litter affects tourism, Krelling et al., 2017). Furthermore, many different actors involved in the development, production, distribution, consumption, regulation and management of plastics are part of the 'plastic system'. These actors directly or indirectly contribute to or are affected by plastic pollution. As a consequence, they have different, often conflicting interests and perspectives that make it difficult to find generally accepted solutions (Kramm and Völker, 2018).

This illustrates that plastic-associated risks are complex, interconnected and of systemic nature which exceeds conventionally applied risk assessment approaches. Complementary, this nature of plastics hampers risk management decisions. For instance, the fact that there is more than one risk producing group makes it difficult to find management strategies compatible with all interests (International Risk Governance Council, 2005). Thus, new concepts for risk description become necessary and innovative research is needed that analyzes today's environmental pollution issues, such as plastics, and proposes solution options.

[11]Risk is here defined as the "*likelihood that an undesirable state of reality (adverse effects) may occur as a result of natural events or human activities*" (Renn, 2008).

1.5 Researching plastics – The PlastX project

Inter- and transdisciplinary research is a kind of such innovative research and can build one pillar in addressing systemic risks and, thus, also plastics in the environment. Transdisciplinary research is a genuine mode of sustainability research that addresses societal problems by the collaboration of different scientific disciplines and extra-scientific actors that play a role in the given problem context. In a three-step approach, it (1) creates a common research object, (2) produces new knowledge by interdisciplinary integration and re-connects it with societal practical knowledge to develop applicable solution and action strategies and (3) finally, assesses the integrated results. As reflective research approach, it also allows mutual learning between science and extra-scientific groups (Jahn et al., 2012).

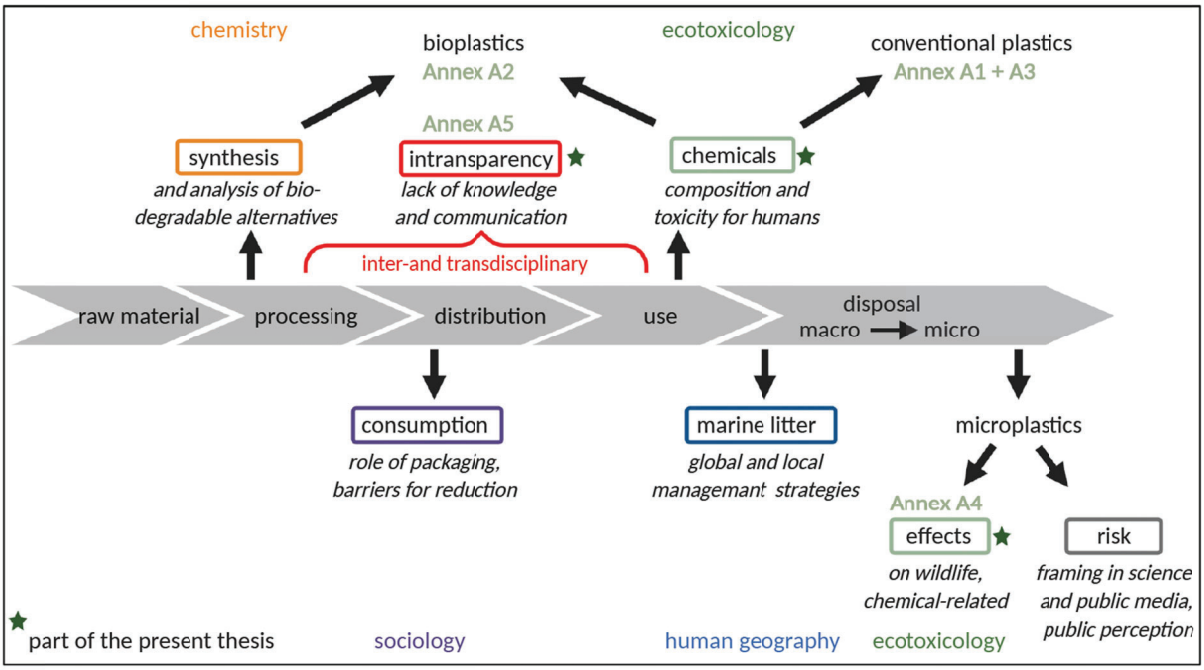

Figure 1. Research fields of the PlastX project and the integration of the present thesis in the project. Scientific disciplines are illustrated with their research focus at the corresponding phases of the plastic life cycle (shown as line for the sake of clarity). The figure was created with Biorender.com.

The research project 'PlastX – researching plastics from a social-ecological risk perspective', as part of which the present thesis was realized, applied a transdisciplinary research approach. The project aimed to unravel the role of plastics in our society and the potential risks of plastics in the environment to finally propose sustainable and applicable solution options. The adoption of a social-ecological risk perspective allowed to consider risks to humans and the

environment (Völker et al., 2017). For a holistic assessment of the complex research object, the project covered several topics along the plastic life cycle that were investigated from four scientific perspectives (chemistry, sociology, human geography and ecotoxicology; Figure 1). Following an inter-and transdisciplinary research mode, the results of the different scientific disciplines were integrated and complemented by the expertise and interests of various practice partners involved in the plastic topic (e.g., plastic industry, consumer protection, environmental consulting, water and waste management). In addition, the project worked at a conceptual level. Here, it aimed to enhance the theoretical understanding of systemic risks in social-ecological systems by integrating natural and social science perspectives. The work and overall approach of PlastX can ultimately serve as a future-oriented example of how to approach systemic risks that characterize the Anthropocene, such as plastics in the environment.

1.6 Aims and structure of the thesis

As outlined above, plastics consist of a multitude of chemicals but knowledge on their overall toxicity and composition remains scarce. Thus, the present PhD thesis aims at a comprehensive toxicological and chemical characterization of the chemical mixtures present in and leaching from a wide range of everyday plastics. Therefore, *in vitro* and *in vivo* toxicity assessments of commonly used plastic products, as well as of microplastics, respectively, were combined with chemical analysis. Besides the generation of experimental data in the discipline of ecotoxicology, the findings were integrated in the PlastX project. Overall, the present body of studies contributes to the safety evaluation of plastics and hazard assessment of microplastics, and, thus, to a holistic understanding of the problems connected to plastics. In addition, this thesis derives solution options for the safer design of plastics and their alternatives as well as recommendations towards a more sustainable plastic economy.

The first three studies (A1–A3) focused on consumer plastics, such as yoghurt cups, plastic wraps, disposable cutlery, drinking and shampoo bottles, since they represent a major exposure source of plastic chemicals to humans and the environment (Figure 2). For a comprehensive and comparative profiling, 77 products were selected in total. These covered

- 16 material types, including seven conventional, petroleum-based materials with the highest market share as well as nine bioplastics and plant-based materials,
- products with and without food contact,
- raw materials (pre-production pellets) and final products.

In order to assess the toxicity and composition of chemical mixtures contained in and released from plastics into water, samples were cut and chemicals leached. The leachates were applied to a set of four *in vitro* bioassays for the analysis of unspecific endpoints (baseline toxicity, induction of an oxidative stress response and cytotoxicity) and endocrine activities (estrogenicity and antiandrogenicity), as well as to non-target high-resolution gas or liquid chromatography mass spectrometry for chemical profiling.

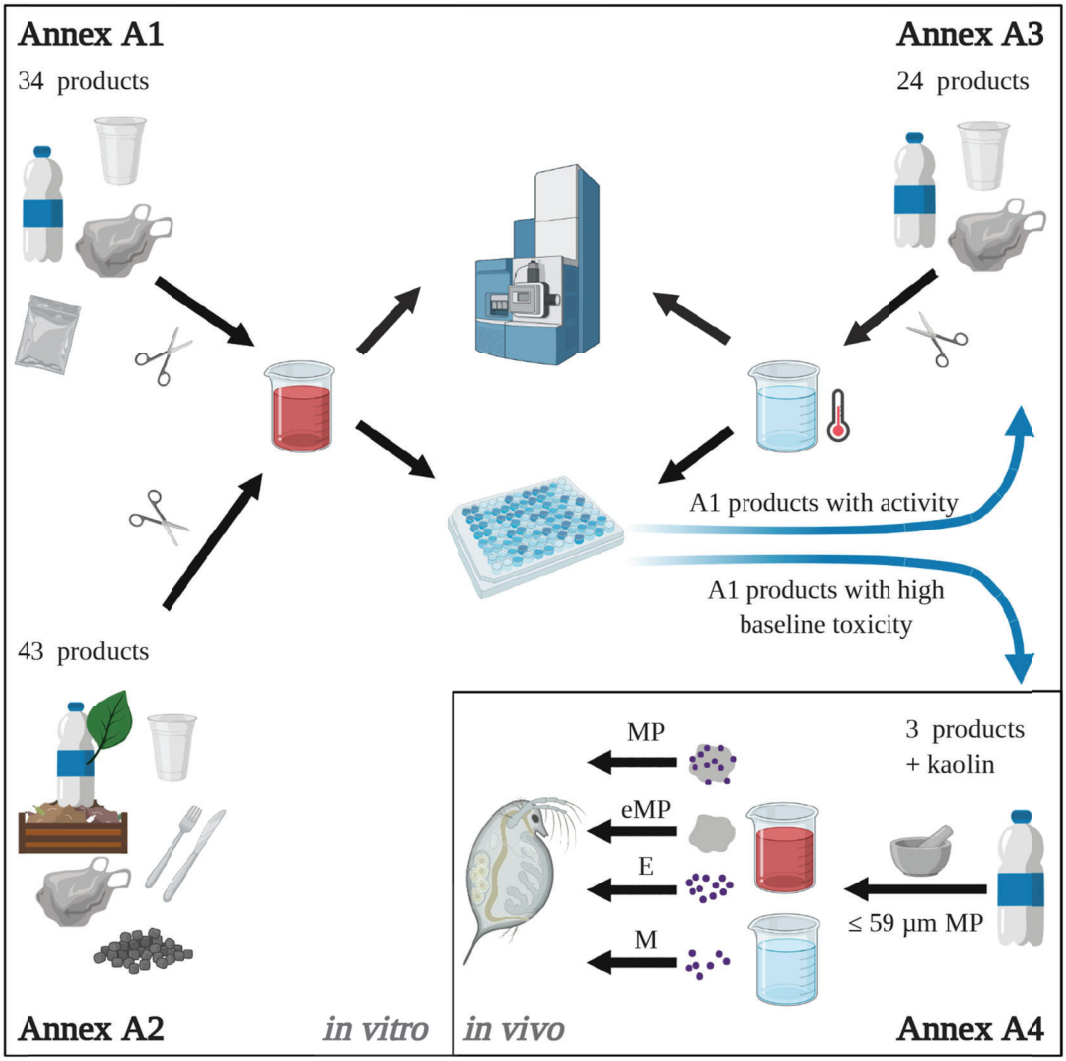

Figure 2. Overview of the four experimental studies of the present thesis including their set-ups and linkages. MP, microplastics; eMP, microplastics extracted with methanol; E, extract, M; migrates. The figure was created with BioRender.com.

1.6.1 *In vitro* toxicity and chemical composition of everyday plastics (A1)

The first study focused on the chemicals present in 34 everyday plastics with and without food contact and made of the petroleum-based polymers with the highest market share, HDPE, LDPE, PS, PP, PET, PVC and PUR, as well as the bio-based and biodegradable PLA. Samples were extracted with methanol to isolate most of the chemicals present in the products. Besides a toxicological profiling of these extracts using *in vitro* bioassays, non-target GC-QTOF-MS/MS shed light on the number and abundance of chemicals and subsequent tentative identification of compounds on their origin, functionality and toxicity. The following hypotheses were evaluated:

- The toxicity present in plastics[12] can be benchmarked based on the polymer type.
- Plastics contain a large variety of chemicals.
- The chemical signature of a plastic product predicts its toxicity.
- FCMs contain less toxicity than non-FCMs.

1.6.2 *In vitro* toxicity and chemical composition of bio-based and biodegradable materials (A2)

Bioplastics and plant-based materials are often considered to be a sustainable alternative to conventional plastics. Since the products made of the bioplastic PLA contained toxic chemicals (A1), this raised the question of whether aspects of chemical safety are neglected in the design of 'better' plastic alternatives. Thus, a set of 43 final products and their precursors, covering nine materials and mostly FCMs, were characterized toxicologically and chemically in a similar way as the conventional plastics before. Feedstock and biodegradability permits grouping of products into bio-based (Bio-PE, Bio-PET), biodegradable (polybutylene succinate, PBS; polybutylene adipate terephthalate, PBAT), both bio-based and biodegradable materials (PLA; Polyhydroxyalkanoates, PHA), as well as plant-based products (starch-, cellulose-, bamboo-blends). In contrast to the first study, UPLC-QTOF-MS/MS was performed to evaluate the chemical similarity within and between material types, as well as to tentatively identify the most prevalent features across all samples and the most abundant features in each sample. The experimental design addressed the following hypotheses:

- Bioplastics and plant-based materials are similarly as toxic as conventional plastics.

[12]For the ease of reading, in some cases 'toxicity contained in/leaching from plastics' is used to refer to the 'toxicity induced by the chemicals contained in/leaching from plastics'.

- Chemical complexity and toxicity increase in processing from the raw materials to the final products.

1.6.3 *In vitro* toxicity and composition of chemical mixtures leaching from plastics under realistic use conditions (A3)

Plastic chemicals and their toxicity will only become available for human and wildlife exposure when released under real-world conditions. Thus, the 24 plastic products that had induced toxicity in the first study (A1) were selected and migration experiments performed with water according to the standard procedures for testing plastic FCMs in the EU (EU, 2011a). After solid phase extraction (SPE) of aqueous migrates, their toxicity was evaluated as before. In addition, *in vitro* effects of these migrates were compared to those of the methanolic extracts of the first study which were produced from the same product. UPLC-QTOF-MS/MS was applied for the chemical characterization of migrates and extracts. The analyses covered the numbers and abundances of the chemicals associated with a single plastic product, the readiness of chemicals to migrate and the identification of compound using three databases. It was hypothesized that

- chemicals present in plastic products are readily leachable under realistic conditions,
- chemicals migrating from plastics into water induce *in vitro* toxicity,
- the toxicity and number of leached chemicals is specific to each product and
- the chemical mixtures leaching from plastics into water are less complex and toxic than those extractable with methanol.

1.6.4 The role of plastic chemicals in microplastic toxicity to *Daphnia magna* (A4)

Since the *in vitro* toxicity studies proved that plastic products contain and release complex and toxic chemical mixtures (A1–A3), it was investigated whether plastic chemicals are the driver for microplastic toxicity. In general, effects of microplastics on aquatic organisms have been studied extensively but research has scarcely differentiated between the physical and chemical toxicities of the heterogeneous material. In addition, certain polymer types such as PUR, fragments (vs. beads), as well as naturally occurring particles as references have been left aside. Thus, two chronic exposure experiments were conducted with *Daphnia magna* to analyze the toxicity of microplastics based on the less-studied polymer types PUR, PVC and PLA which were produced from the products that had induced a high *in vitro* toxicity in the

first study (Table 1; A1). The investigations focused on mortality, reproductive output, timing of reproduction and body lengths of the pelagic freshwater invertebrate. The first experiment with 10, 50, 100 and 500 mg L^{-1} of irregular microplastics and natural kaolin particles (all ≤ 59 µm) tested the hypothesis that

- PUR, PVC and PLA microplastics affect life-history traits of *D. magna,*
- toxicity changes with the plastic type and
- natural kaolin particles are less toxic than microplastics.

The second experiment compared the effects of untreated microplastics and microplastics free of extractable chemicals, as well as the extractable chemicals (worst-case scenario) and the chemicals migrating into water (realistic scenario) to test the hypothesis that plastic chemicals are the driving factor for microplastic toxicity.

1.6.5 Lacks in transparency connected with plastic packaging (A5)

During the acquisition of products used in the present work and in correspondence with producers and retailers, a lack of knowledge and knowledge transfer regarding plastic ingredients were observed. These deficiencies were also noticed in ethnographic-sociological research (by Lukas Sattlegger, PlastX) and in entrepreneurial practice (by Maik Birnbaum, head of sustainable packaging at einhorn products GmbH) but from different viewpoints. Following a transdisciplinary approach, the different scientific and economic experiences and perspectives on the topic were integrated. The aim of the resulting publication was not to test for hypotheses but to derive recommendations for appropriate action based on the observations, which was done in a subsequent step. These recommendations are directed to political decision makers, economic actors and sustainability researchers who can help to overcome these issues and arrive at safer and more sustainable packaging.

2 Discussion

In the following, the main findings of this PhD thesis are briefly summarized (2.1). Detailed information on the experimental design and results can be found in the respective publications in A1–A5. The subsequent chapters comprise the experimental results and discuss the toxicity and chemical composition of conventional plastic products (2.2) and their bio-based and biodegradable alternatives (2.3), in addition to the drivers of microplastic toxicity (2.4), in the broader context of human and environmental health. Furthermore, the experimental findings are integrated in the social-ecological research context of the PlastX project to elaborate on intransparency issues connected with plastics and their chemicals (2.2) as well as on risks of plastics and microplastics (2.5). Finally, measures to improve the safety of materials and to support a more sustainable plastic economy are proposed (2.6).

2.1 Key findings of the present work

The comprehensive toxicological and chemical characterization of conventional plastics and their alternatives highlights that

1. Plastic consumer products contain chemicals that trigger *in vitro* toxicity. The toxicity of bio-based and biodegradable materials is comparable to that of conventional petroleum-based plastics (Table 1; A1, A2).

- 67% of the 30 petroleum-based plastics induced *in vitro* toxicity, including baseline toxicity (57%), oxidative stress (43%), cytotoxicity (30%), estrogenicity (13%) and antiandrogenicity (30%; A1).
- 67% of the 43 bioplastics and plant-based materials induced *in vitro* toxicity, including baseline toxicity (67%), oxidative stress (42%), estrogenicity (2%) and antiandrogenicity (23%; A2).
- Unspecific toxicity (baseline toxicity, oxidative stress response and cytotoxicity) was more frequent than antiandrogenicity which was, itself, more prevalent than estrogenicity (A1, A2).

2. Plastics, as well as their bio-based and biodegradable alternatives, contain a wide variety of chemicals, many of which are unknown.

- In conventional plastics, a total of 1411 chemical features were detected but only 260 compounds (18%) were tentatively identified (A1).
- Altogether, the 43 bioplastics and plant-based materials contained 41,395 chemical features where 80% of the individual samples contained > 1000 and up to 20,000 chemicals. Most chemicals were unique to one sample (A2).
- The chemical complexity does not predict the toxicity of a product (A1, A2).
- Tentatively identified chemicals include plastic-associated compounds, such as monomers, oligomers, additives, solvents, lubricants, process regulators and further NIAS, as well as compounds originating from the packaged content (A1, A2).

3. Under realistic conditions, plastic products readily leach many chemicals that trigger *in vitro* toxicity and are mostly unknown (A3).

- All 24 migrates induced baseline toxicity, 22 activated an oxidative stress response, 13 had an antiandrogenic activity and one an estrogenic activity.
- Generally, the aqueous migrates triggered a lower *in vitro* toxicity than the methanolic extracts, thus, not all toxicity contained in a product is released. However, some migrates were as toxic as the extracts.
- A total of 685 to 17,973 unique chemicals features were detected in a single plastic product (i.e., in the migrate and extract). Out of these, between 17 and 8572 chemical features were readily leachable and up to 1612 were only detected in the migrate.
- Only 3% of all detected features were tentatively identified.

Taking these studies together demonstrates the following:

4. Not all plastics are alike: every product has an individual chemical composition and toxicity. This prevents a generalization of the latter aspects based on material type, feedstock, and biodegradability, as well as the processing state of the product or the product's use with or without food contact.

- While some material types (PVC, starch- and cellulose-blends) generally contained and leached more chemicals and a higher toxicity than others (HDPE, PET, bamboo-blend and Bio-PE), all material types comprised toxic products (A1–A3).[13]
- The percentage of bio-based and biodegradable samples inducing toxicity was identical to conventional plastics (67%) and mean effect strengths of both groups were comparable. In addition, toxicity was as prevalent in PE products made from renewable feedstocks as in PE products made from petroleum (A2).
- Generally, toxicity and chemical complexity of plastics increased with processing since toxic chemicals were less prevalent and potent in the raw materials than in the final products. However, also two pre-production pellets induced a similarly high baseline toxicity as the most toxic final product (A2).
- Overall, chemicals in non-FCMs triggered a higher toxicity, but some FCM samples induced a similar or even higher toxicity than non-FCM samples (A1, A3).

The main findings of the chronic exposure experiments with the freshwater crustacean *D. magna* and microplastics (A4) are:

5. Microplastic fragments based on PVC, PUR and PLA affect life-history traits of *D. magna*.
    - The toxicity of microplastics was dependent on the material and the endpoint; PVC reduced reproduction the most and PLA induced the highest mortality.
    - Microplastics based on bio-based and biodegradable PLA were as toxic as microplastics made from petroleum.
    - The natural kaolin particles were less toxic than all tested microplastic types.
6. Plastic chemicals can drive microplastic toxicity.
    - While for PVC the chemicals used in the material induced the effects, for PUR and PLA it was the mere particle.

The experimental findings and personal observations gained during this work, integrated with the perspectives of a social scientist and an entrepreneur, elucidated that:

7. Knowledge on plastic packaging and its chemical composition remain inaccessible to many actors along the whole product life cycle due to different reasons:
    - There is a general lack of knowledge regarding the chemical composition of plastics. For instance, many substances formed during production are unknown.

[13]With the exception of the bamboo-based sample that did not induce toxicity. However, the analysis included only a single product made of bamboo.

- The existing knowledge is kept concealed or is insufficiently transferred, i.e., intransparent. For instance, manufacturers are not obliged to disclose the chemical composition of their products. Thus, product ingredients remain unknown for other actors, such as product manufacturers or the public.
- The available knowledge is not applicable or connectable, i.e., incompatible. For instance, life cycle assessments (LCAs) are highly context dependent. This complicates their application on concrete processes, such as product development, which impedes safe product design.

Recommendations to address these issues and to promote a collaborative development of safe and sustainable packaging were derived from the studies and are described in chapter 2.7.

Table 1. Overview of samples analyzed in the studies A1–A4 with their mean effects in bioassays.

		Annex A1						Annex A3				
		unspecific toxicity	oxidative stress	EA	AA			unspecific toxicity	oxidative stress	EA	AA	
plastic category	polymer type	sample name	EC_{20} (mg plastic)[a]	EC_{IR2} (mg plastic)[b]	rEA(%)[c]	rAA (%)[d]	FCM[e]	product	product category[f]	sample name	EC_{20} (mg plastic)[g]	EC_{IR2} (mg plastic)[h]	rEA (%)[i]	rAA (%)[j]
petroleum-based, non-biodegradable	HDPE	HDPE 1	—	—	0.08	4.73	yes	refillable drinking bottle	drinking bottle	x				
		HDPE 2	—	—	0.62	0.20	yes	yoghurt drink bottle	yoghurt cup	x				
		HDPE 3	14.6	—	2.75	31.5	no	bin liner	foil	HDPE 1	53.2	—	0.51	92.1
		HDPE 4	—	—	0.04		no	shower gel bottle	shampoo bottle	x				
	LDPE	LDPE 1	4.34	—	0.28	2.16	yes	juice bottle	drinking bottle	LDPE 1	2.55	12.0	—	32.6
		LDPE 2	1.02	—	2.21	9.30	yes	plastic wrap	foil	LDPE 2	2.83	60.6	1.04	—
		LDPE 3	—	—	1.63	11.1	yes	freezer bag	bag	LDPE 3	22.6	64.8	0.75	71.6
		LDPE 4	2.63	0.48	0.71	12.3	no	hair conditioner bottle	shampoo bottle	LDPE 4	3.38	2.15	0.50	91.5
	PS	PS 1	—	—	3.82	—	yes	yoghurt cup	yoghurt cup	PS 1	17.7	9.64	—	61.6
		PS 2	1.30	2.78	1.13	—	yes	fruit tray	tray	PS 2	0.48	87.3	0.82	—
		PS 3	22.3	—	0.37	—	yes	vegetable tray	tray	PS 3	321	97.2	0.14	0.72
		PS 4	18.3	—	1.17	1.16	yes	plastic cup	other FCM	PS 4	120	107	0.84	7.07
	PP	PP 1	—	—	0.10	11.3	yes	refillable drinking bottle	drinking bottle	x				
		PP 2	—	3.83	—	47.8	yes	yoghurt cup	yoghurt cup	PP 1	22.2	3.20	—	40.6
		PP 3	3.63	0.99	—	—	yes	gummy candy packaging	foil	PP 2	4.24	7.55	—	—
		PP 4	—	—	0.22	9.97	no	handkerchief packaging	foil	x				
		PP 5	8.00	—	0.22	—	no	shampoo bottle	shampoo bottle	x				
	PET	PET 1	—	—	—	2.79	yes	soft drink bottle	drinking bottle	x				
		PET 2	—	—	—	12.9	yes	yoghurt cup	yoghurt cup	x				
		PET 3	—	6.69	1.22	16.3	yes	oven bag	bag	PET 1	10.6	131	—	1.13
		PET 4	—	—	0.13	4.57	yes	vegetable tray	tray	x				
		PET 5	—	—	0.21	2.66	no	shampoo bottle	shampoo bottle	x				
	PVC	PVC 1	0.23	6.64	—	25.5	yes	plastic wrap	foil	PVC 1	0.56	6.62	0.46	54.0
		PVC 2	1.22	2.14	27.1	86.8	no	place mat	other non-FCM	PVC 2	0.14	91.6	59.4	90.9
		PVC 3	1.80	2.42	—	34.6	no	pond liner	other non-FCM	PVC 3	0.93	74.0	0.21	—
		PVC 4 ★	0.49	1.16	6.91	48.4	no	floor covering	other non-FCM	PVC 4	0.12	18.8	0.98	19.3
	PUR	PUR 1 ★	3.02	1.13	0.59	55.8	no	scouring pad	other non-FCM	PUR 1	23.2	25.0	0.01	45.9
		PUR 2	2.73	0.51	—	69.0	no	kids bath sponge	other non-FCM	PUR 2	2.04	12.9	0.36	65.8
		PUR 3	3.56	0.47	0.36	82.3	no	acoustic foam	other non-FCM	PUR 3	32.7	15.2	0.90	79.9
		PUR 4	10.2	1.82	2.26	30.4	no	kids bath sponge	other non-FCM	PUR 4	2.63	19.5	0.43	92.2

			unspecific toxicity	oxidative stress	EA	AA					unspecific toxicity	oxidative stress	EA	AA
plastic category	polymer type	sample name	EC_{20} (mg plastic)[a]	EC_{IR2} (mg plastic)[b]	rEA(%)[c]	rAA (%)[d]	FCM[e]	product	product category[f]	sample name	EC_{20} (mg plastic)[g]	EC_{IR2} (mg plastic)[h]	rEA (%)[i]	rAA (%)[j]
bio-based, biodegradable	PLA	PLA 1	2.12	—	0.02	—	yes	yoghurt cup	yoghurt cup	PLA 1	109	104	—	42.5
		PLA 2	6.21	—	0.34	13.2	yes	vegetable tray	tray	PLA 2	47.4	111	—	7.85
		PLA 3★	0.01	0.98	—	—	no	shampoo bottle	shampoo bottle	PLA 3	3.98	26.9	—	4.51
		PLA 4	1.32	—	—	15.3	yes	coffee cup lid	other FCM	PLA 4	143	—	—	12.3

Annex A2

	polymer type	sample name	EC_{20} (mg plastic)[a]	EC_{IR2} (mg plastic)[b]	rEA(%)[k]	rAA (%)[m]	FCM[e]	product	product category[f]	processing state[n]
bio-based, biodegradable	PLA	PLA 1	18.4	—	0.20	4.41	yes	single-use drinking cup	other FCM	P
		PLA 2	—	—	0.28	18.9	yes	disposable cutlery	other FCM	P
		PLA 3	12.4	—	2.49	18.2	yes	film	foil	P
		PLA 4	1.81	—	0.26	—	yes	food tray	tray	P
		PLA 5	1.61	1.12	1.29	18.8	yes	coffee capsule	other FCM	P
		PLA 6	4.91	—	—	69.9	yes	bag for foodstuff	bag	P
		PLA 7	—	6.76	0.16	5.34	yes	single-use bottle	drinking bottle	P
		PLA 8	4.73	—	0.29	8.88	no	film	foil	P
		PLA 9	—	—	0.04	0.69	yes	pellet	—	RM
		PLA 10	—	—	0.15	—	no	pellet	—	RM
	PHA	PHA 1	0.60	—	—	—	no	pellet	—	RM
plant-based blend	starch	Starch 1	18.6	—	0.45	—	yes	disposable cutlery	other FCM	P
		Starch 2	1.22	3.20	0.09	38.1	yes	bag for foodstuff	bag	P
		Starch 3	0.29	5.98	0.15	80.8	no	film	foil	P
		Starch 4	2.81	4.90	—	38.0	yes	film	foil	P
		Starch 5	6.80	—	0.23	21.4	yes	pellet	—	RM
		Starch 6	18.9	7.37	0.21	4.50	yes	pellet	—	RM
		Starch 7	0.69	—	—	57.3	no	waste bag	bag	P
		Starch 8	5.75	—	—	41.5	no	film	foil	P
	cellulose	Cellulose 1	1.04	2.18	0.70	78.5	yes	tea bag wrapping	foil	P
		Cellulose 2	1.63	6.77	0.75	1.06	yes	chocolate wrapping	foil	P
		Cellulose 3	0.69	6.76	0.01	33.4	no	cigarette filter	other non-FCM	P
		Cellulose 4	1.39	7.19	0.04	19.3	yes	pellet	—	RM
		Cellulose 5	2.61	6.00	0.24	77.9	yes	bag for foodstuff	bag	P
		Cellulose 6	1.20	6.07	—	—	yes	bag for foodstuff	bag	P
		Cellulose 7	2.42	—	—	1.49	yes	bag for foodstuff	bag	P
	bamboo	Bamboo1	—	—	—	—	yes	reusable coffee cup	other FCM	P

Annex A4 Reproduction

Treatment	EC_{50}[p] (mg l^{-1})	EC_{50}[p] (particle l^{-1})
PVC	45.5	1.14×10^7
PUR	236	7.29×10^7
PLA	122	5.13×10^7
kaolin	275	2.61×10^9

bio-based, non-biodegradable	Bio-PE	Bio-PE 1	16.3	6.66	—	91.3	yes	bag for foodstuff	bag	P
		Bio-PE 2	—	7.01	0.11	6.80	yes	wine closure	other FCM	P
		Bio-PE 3	5.01	6.24	—	45.2	yes	bag for foodstuff	bag	P
		Bio-PE 4	—	—	0.40	10.8	no	pellet	—	RM
		Bio-PE 5	—	—	0.15	—	yes	food tray	tray	P
		Bio-PE 6	—	—	0.13	—	no	film	foil	P
		Bio-PE 7	—	—	0.24	2.03	yes	wine closure	other FCM	P
		Bio-PE 8	—	—	0.01	—	no	pellet	—	RM
		Bio-PE 9	0.35	3.60	—	97.4	yes	bag for foodstuff	bag	P
		Bio-PE 10	8.14	—	—	45.9	yes	film	foil	P
	Bio-PET	Bio-PET 1	—	—	0.37	—	yes	reusable bottle	drinking bottle	P
		Bio-PET 2	1.85	0.58	0.63	52.6	no	bag	bag	P
petroleum-based, biodegradable	PBS	PBS 1	—	—	0.51	8.11	no	plastic bar	—	RM
		PBS 2	12.9	7.07	0.40	7.22	yes	food tray	tray	P
	PBAT	PBAT 1	5.69	—	0.01	98.0	yes	waste bag	bag	P
		PBAT 2	—	—	0.11	26.5	yes	pellet	—	RM

Note: (r)EA, (relative) estrogenic activity; (r)AA, (relative) antiandrogenic activity; —, no activity observed; x, not analyzed; ★, used for experiments in A4;

[a] mg plastic extracted leading to 20% luminescence inhibition (EC_{20}), concentrations up to 22.5 mg plastic well^{-1} were tested.

[b] effective concentration (EC) in mg plastic extracted leading to a luciferase induction ratio of 2.0 over the control (IR 2), concentrations up to 7.5 mg plastic well^{-1} were tested.

[c] rEA for 3.75 mg plastic or the highest measured non-cytotoxic concentration, limit of detection (LOD): 2.33%.

[d] rAA for 3.75 mg plastic or the highest measured non-cytotoxic concentration, LOD: 29.2%.

[e] food contact material.

[f] the application category the product was assigned to (see also Figure 4).

[g] concentrations up to 600 mg plastic well^{-1} were tested.

[h] concentrations up to 200 mg plastic well^{-1} were tested.

[I] rEA for 100 mg plastic or the highest measured non-cytotoxic concentration, LOD: 1.65%.

[j] rEA for 100 mg plastic or the highest measured non-cytotoxic concentration, LOD: 27.3%.

[k] rEA for 3.75 mg plastic or the highest measured non-cytotoxic concentration, LOD: 1.56%.

[m] rEA for 3.75 mg plastic or the highest measured non-cytotoxic concentration, LOD: 48.3%.

[n] raw material (RM), final product (P).

[p] concentrations reducing the reproduction of *Daphnia magna* by 50% (EC_{50}).

Red coloring marks samples with toxic effect, meaning an effect above the set threshold for the respective endpoint. Effect thresholds are an EC_{20} for baseline toxicity, an EC_{IR2} for oxidative stress response and the LOD for endocrine endpoints. Green coloring marks samples without toxic effect on that endpoint.

2.2 Toxicological and chemical characterization of plastic products

With the rise of the plastic age, negative side effects of plastic use became also evident. The public debate on human health concerns as well as research have largely revolved around single 'plastic-typical' chemicals, such as BPA and phthalates. However, to assess the hazards of plastics, all chemicals making up the material need consideration. Following this train of thoughts, in the present thesis the focus was set on the overall chemical mixtures and an initial toxicological benchmarking of everyday plastics was performed.

2.2.1 *In vitro* toxicity of plastics

As a first step, the chemical mixtures contained in 77 everyday plastics were characterized for their *in vitro* toxicity. Plastics were extracted with methanol to isolate most of the chemicals present in the material[14] without dissolving it. The majority of products (71%) contained chemicals inducing at least one of the analyzed endpoints, including cytotoxicity, baseline toxicity, oxidative stress response, antiandrogenicity and estrogenicity (Table 1; A1, A2).

Since humans and wildlife will only be exposed to these compounds and their toxicities when they are actually released under realistic conditions, the next study analyzed the chemicals migrating into water. Strikingly, all 24 products leached mixtures that affected at least one of the *in vitro* endpoints (Table 1; A3). These results are in line with previous research on the same polymer types and endpoints reporting that plastic products leach compounds inducing unspecific toxicity (e.g., Ramot et al., 2016: PLA applications in medicine), cytotoxicity (e.g., Sauvant et al., 1995: PET and PVC resins and bottles), estrogenic (e.g., Wagner and Oehlmann, 2009: PET water bottles) as well as antiandrogenic effects (e.g., Mertl et al., 2014: different FCMs) under realistic use conditions. In the literature, *in vitro* data on plastic chemicals inducing an oxidative stress response is limited to weathering experiments with microplastics (Rummel et al., 2019). However, plastic chemicals that trigger an oxidative stress response seem be widespread across plastic products. Accordingly, 32 out of the 77 extracts (A1, A2) and 22 out of the 24 migrates (A3) induced an oxidative stress response (Table 1).

The pair-wise comparison of the aqueous migrates with the methanolic extracts of the same sample indicates that toxic chemicals readily migrate into water. For instance, half of

[14]In the following, 'present/contained in' refers to the chemicals (and their toxicity) extractable with methanol since it will dissolve most chemicals contained in the material, while 'leaching/migrating' refers to those released into water if not stated otherwise.

the migrates induced a similar or higher baseline toxicity as their corresponding extracts. Interestingly, chemicals triggering oxidative stress or endocrine activities had mostly a lower migration potential (see 2.2.2). This means that the migrate was less toxic on these endpoints than the extract. However, exceptions were observed for some products whose migrates were as potent as their extract (A3). Overall, this demonstrates that plastic products do not only contain but also readily release toxic chemicals under realistic conditions. In this way, humans and wildlife become actually exposed to them.

Some migrate samples analyzed in the present work triggered *in vitro* toxicities already at low concentrations and with a high potency. As an example, chemicals leaching from less than 1 mg plastic triggered 20% bioluminescent inhibition in the case of four PVC and one PS product as well as 50% estrogenicity and antiandrogenicity in the case of one PVC sample (Table 2; A3). It appears plausible that humans and wildlife are actually exposed to such concentrations when considering daily plastic use as well as the amounts of litter in the environment.

The applied bioassay-based approach integrates effects of all the compounds present in the mixture and seems to be a powerful tool to deal with the chemical complexity of plastics. Therefore, the approach can be used to receive a first benchmarking in the risk evaluation of these chemical mixtures. To assess human health risks, effects on higher levels of biological organization have to be evaluated that take into account the toxicokinetic processes and interactions present within a complex multicellular organisms (Groh and Muncke, 2017). In addition, information on the actual exposure levels is required. Here, it has to be kept in mind that plastics are only one source of exposure to their associated chemicals (Muncke et al., 2020).

2.2.2 Differentiation between unspecific and endocrine effects

In this thesis, effects on two unspecific (baseline toxicity, oxidative stress induction) and two endocrine endpoints (estrogenic and antiandrogenic activity) were investigated. While baseline toxicity indicates unspecific effects relevant in the environmental context, the remaining indicate effects relevant for human health. The results demonstrate that chemicals triggering an unspecific toxicity are more prevalent in plastic extracts and migrates than EDCs (A1–A3). This in line with a study of Szczepańska et al (2016) reporting a widespread induction of baseline toxicity by plastic migrates. The prevalence of baseline toxicity might be linked to the fact that many compounds cause effects on this endpoint (Neale et al., 2012).

Additionally, the present work points out that antiandrogenic compounds are more frequently contained in plastics than estrogenic compounds. At the same time, the samples' antiandrogenicity was more potent (A1, A2). A similar picture was previously observed for PP, PE and PS products (Mertl et al., 2014). In contrast, Yang et al. (2011) analyzed 455 plastic food containers and, using the E-Screen, detected estrogenicity in most of them. The higher prevalence of estrogenicity in the study of Yang et al. may be explained by different sensitivities of the YES assay (used here) and the E-Screen. Mertl et al. (2014) compared the sensitivity of the YES with the CALUX assay, that is, like the E-Screen, based on a mammalian cell line. The authors observed a lower sensitivity of the YES while the assays' specificities were comparable. Wagner and Oehlmann (2011) also reported a lower sensitivity of the YES when comparing it to the E-Screen. This may indicate that assays based on human cell lines generally have a higher sensitivity for estrogens than yeast-based assays (which would explain the higher prevalence of estrogenicity in the study of Yang et al.). Mertl et al. (2014), additionally, compared antagonistic activities of the YAAS and the CALUX. They observed more samples with effects in the former and concluded that these antagonistic activities resulted of yeast-specific interactions and, thus, that the YAAS can lead to false positive results. However, observed antagonistic effects in the YAAS were receptor-specific and unspecific effects could be excluded (Mertl et al., 2014). Hence, the yeast-based assay may also have a higher sensitivity for detecting androgen receptor antagonists in complex chemical mixtures than the CALUX. Generally, there are different opinions whether only human cell-based assays are appropriate to predict human health effects or whether yeast-based assays may even have a higher sensitivity in detecting estrogen and androgen receptor-antagonists (Groh and Muncke, 2017). Further investigations are necessary to clarify this issue. When interpreting and comparing results of *in vitro* assays, it has to be kept in mind that sample preparation and extraction techniques strongly influence the absolute recovery of chemicals present in the sample (e.g., due to loss of volatile compounds or inefficient enrichment of target compounds) and therefore also measured activities. In addition, the presence of antagonists may mask effects of agonists (Wagner and Oehlmann, 2011). Along that line, the above described low prevalence of estrogenicity in the present study may be due the presence of antiestrogens masking effects of estrogens. The testing and comparing of both agonistic and antagonistic activities would help to resolve that matter.

Putting these discussions aside, it is remarkable that several studies reported that plastics leach chemicals with endocrine activity (e.g., Berger et al., 2015; Kirchnawy et al., 2014; Wagner and Oehlmann, 2009). This emphasizes the importance to consider endocrine

endpoints in regulations such as the one for plastic FCMs, especially, when also keeping in mind that migrates can be effective at low concentrations (A3; EU, 2011a; Muncke et al., 2017). This necessity is underlined by the recently published review by Vinggaard et al. (2020). The review demonstrates that chemicals inducing estrogenic and antiandrogenic activities *in vitro* were commonly present in human samples (e.g., blood, urine) and that these activities were associated with breast cancer and male reproductive health disorders. This emphasizes the great potential of *in vitro* tests to uncover human health hazards holistically and to identify the molecular events which may initiate a disease later in life.

2.2.3 Toxicity by product categories

In case product categorization schemes (e.g., based on the polymer type) would indicate the toxicity of a product, it would be possible to give recommendation on how to reduce use of and exposure to toxic plastic chemicals. These recommendations could guide industry in product development and processing, vendors and consumers in product choice, as well as decision makers in the elaboration of regulations and practical measures. The present thesis covers a multitude of products which permits comparisons between different product categories. Products can be categorized according to four schemes: (1) polymer type, (2) food contact, (3) application and (4) processing state (Table 1). The comparative analyses demonstrate that these categories barely allow for a general statement on the toxicities of products belonging to them (A1–A3).

(1) Polymer type: The *in vitro* toxicity assessments illustrate that PVC and PUR products more frequently contain and leach chemical mixtures of high toxicity (A1, A3). Based on the hazard of their chemical monomers and since they require particularly high quantities of additives, PVC and PUR have already been ranked as the most hazardous polymers before (Lithner et al., 2011). Several *in vivo* studies with aquatic algae and invertebrates underline the higher prevalence of toxic chemicals in products made of PVC or PUR compared to other polymer types (Li et al., 2016; Lithner et al., 2009). This also indicates that the adverse effects are not limited to cells but that plastic chemicals can affect whole organisms. At the other end of the spectrum, PET and HDPE leachates triggered no or low *in vitro* effects (A1, A3). However, it is not possible to make a prediction on the safety of a product based on the polymer type it is made of. Accordingly, there was a least one product within each polymer type that was toxic regarding one or more of the tested endpoints (A1, A3). The literature reflects this diverse picture; some studies reported the absence of toxicity in certain polymer

types, such as of unspecific toxicity (Ceretti et al., 2010), cytotoxicity (Bach et al., 2013) and endocrine activity (Kirchnawy et al., 2014; Mertl et al., 2014) in PET leachates. Other studies observed no toxicity for all polymer types they tested, e.g., no unspecific toxicity of PP, PP and PS pellet leachates (Schiavo et al., 2018). Again other studies detected *in vitro* toxicity across all analyzed polymer types (including PET), although not necessarily for all products (Yang et al., 2011).

(2) Food contact: For the products analyzed in the present work, chemicals of non-FCMs generally induced a higher toxicity than of FCMs. However, chemical mixtures of individual FCMs were similarly, or even more, effective on unspecific or antiandrogenic endpoints than non-FCMs (Figure 3; A1, A3). This underpins previously raised concerns that the current regulation for plastics with food contact is not adequate to assess the safety of plastic FCMs and does not protect human health (EU, 2011b; Groh and Muncke, 2017). As an example, the regulation only prescribes to evaluate the migration of starting substances, neglecting the chemical mixture in the end product (see 2.6.4).

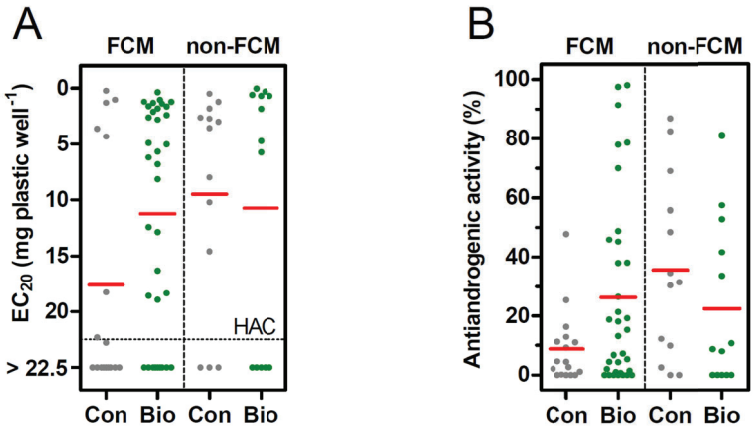

Figure 3. Toxicity of extracts from conventional plastics (Con) as well as bio-based and biodegradable materials (Bio) divided into food contact materials (FCM, Con: n = 17, Bio: n = 34) compared to non-food contact materials (non-FCM, Con: n = 13, Bio: n = 13). (*A*) Effect concentration inducing 20% baseline toxicity. (*B*) Relative antiandrogenic activity from 3.75 mg plastic or, if cytotoxic, for the highest non-cytotoxic concentration (see A1, A2 for details). Each dot represents one extract analyzed in three independent experiments performed in (*A*) duplicates or (*B*) eight replicates and red lines the group means. HAC, highest analyzed concentration.

(3) Product application (e.g., cups to package yoghurt, Table 2): The categorization based on application demonstrates that even if serving the same purpose and being visually identical, the *in vitro* toxicity of plastic chemicals is diverse and each product class can comprise toxic items (Figure 4; A1). Or, if viewed positively, products free of toxic chemicals exist for most applications. Thus, the design of safe products, not containing or not releasing problematic compounds, is possible (A1–A3). Viewed negatively, it is impossible for the general public to identify and to choose non-toxic items. This results in an involuntary and continuous exposure of consumers to potentially hazardous chemicals.

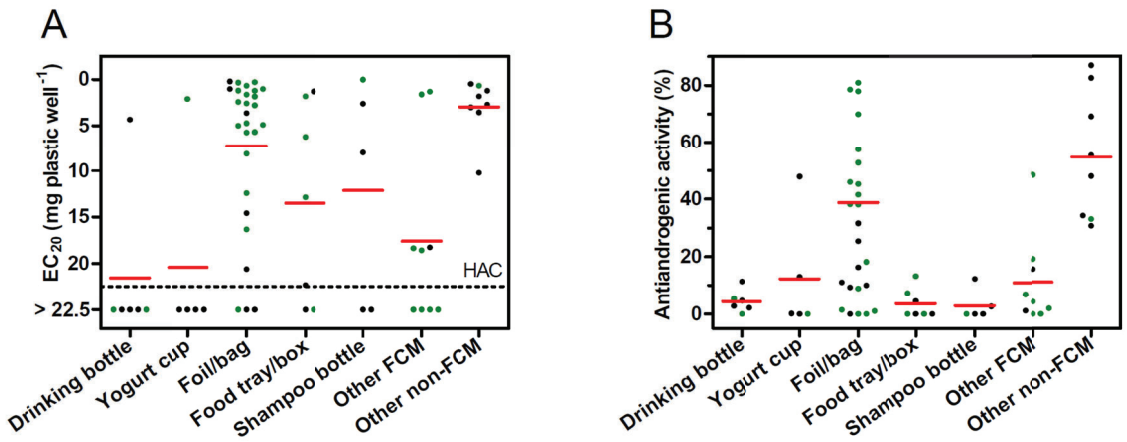

Figure 4. Toxicity of extracts from conventional plastics (black dots) as well as bio-based and biodegradable materials (green dots) categorized by the application of the product. (*A*) Effect concentration inducing 20% baseline toxicity. (*B*) Relative antiandrogenic activity from 3.75 mg plastic or, if cytotoxic, for the highest non-cytotoxic concentration (see A1, A2 for details). Each dot represents one extract analyzed in three independent experiments performed in (*A*) duplicates or (*B*) eight replicates and red lines the group means. HAC, highest analyzed concentration.

(4) Processing state: The results of this thesis imply that during processing and compounding additional chemicals and toxicity are introduced. Accordingly, the mean effect levels and the prevalence of toxicity were twice as high in the final products as in the raw materials. Besides, the number of chemicals increased with processing (Table 1; A2). However, exceptions do exist, for example in the extrusion of pellets (Bio-PE-PLA blends) to film no new compounds were generated (Aznar et al., 2019). This indicates that some processing techniques do not introduce new chemicals. Consequently, a step-wise analysis of the plastic production process can help to identify and abolish the compounding or processing steps in which toxic chemicals are generated. As discussed above, it strongly depends on the respective product, to which extent the toxic chemicals contained in a plastic also leach from

it (2.2.1; A3). Hence, product design can reduce the mobility and the release of inherent chemicals. Strategies to support this aim include the covalent binding of additives to the polymer backbone, the increase of the additives' molecular weights, the change of polymerization towards a lower diffusion coefficient of chemicals or the introduction of inert barriers (Beach et al., 2013; Seidensticker et al., 2019).

2.2.4 Toxicity of bio-based and biodegradable products

It becomes apparent that no real recommendation can be given based on the categories discussed above. In the further search for chemically safer materials, the present work also evaluated bio-based and biodegradable products as alternatives to petroleum-based plastics. The findings provide evidence that these 'biomaterials' not necessarily perform better. Instead, the toxicological heterogeneity between products and within polymer types resembles that of conventional plastics (Table 1). Moreover, the comparison of petroleum and bio-based PE showed that for both feedstock sources half of the products contained toxic chemicals. In addition, both sample types were comparable effective in triggering *in vitro* toxicity (A2). This points out three things: (1) Changing the carbon source of the monomer from petroleum to a plant-based origin will only minimally change the chemical composition and, thus, toxicity of the polymer. (2) Aspects of chemical safety seem not to get special attention in the latest development of 'better' plastic alternatives. (3) Marketing as 'sustainable' does not extend to (chemical) safety (see 2.3 for details).

2.2.5 Chemical complexity of plastics

The question arises whether the chemical profile may explain the discussed toxicities of plastics. Previous analytical studies investigating plastic chemicals have mostly focused on individual products or single compounds and have applied a targeted analysis approach. For a more comprehensive chemical profiling, in the present work, extracts and migrates of the 77 products were applied to high-resolution non-target chemical analysis. The mass spectrometry data indicates that plastic products are composed of a large number of chemicals. Accordingly, individual petroleum-based products contained almost 200 compounds (A1), whilst single bio-based and biodegradable materials contained more than 20,000 compounds (A2). The higher number of detected chemicals in the latter study can be attributed to the different instrumentation: In the first study GC-QTOF-MS/MS was applied to analyze semi-volatile chemicals and in the second study LC-QTOF-MS/MS to analyze non-volatile

compounds. Moreover, deviations in applied settings and data analysis strategies make a direct comparison of the two studies impossible (A1, A2). In order to allow for a direct comparison of compounds contained in and leaching from a plastic product, the non-volatile chemicals in 24 corresponding extracts and migrate samples were analyzed by LC-QTOF-MS/MS (A3). Here, between 613 and 10,766 chemical features were associated with a single plastic product. In addition, the results indicate that plastic chemicals are readily released. Accordingly, up to 84% (PUR product) and on average 29% of the non-volatile compounds detected in a product readily leached into water. This corresponds to high numbers of up to 8936 in the case of one PUR product. A further 5% of all chemical features were only present in the migrates (A3). This suggests a preferential migration of these chemicals into water over methanol or the formation of new compounds during the migration process.

The handful of studies that have used non-target approaches to analyze plastic chemicals, have rarely reported the number of detected features. As an exception, Qian et al. (2018) performed a comprehensive GC-based analysis of 120 plastic FCMs made of seven polymer types and reported dozens or hundreds of different components per sample. In a non-target LC-QTOF-MS/MS screen on 104 honey samples stored in plastic or glass jars 104,051 molecular features were detected in total (Eyken et al., 2020). Ubeda et al. (2019b) did not report the total number of chemical features but made a similar comparison as in the present work by comparing total extract (using dichloromethane as solvent) and migrates (using different food simulants) of PLA-PE-blends. The authors identified 15 compounds in extracts and only two in migrates. These results support the observation of the present thesis that a single plastic product can contain and release a multitude of chemicals.

2.2.6 Product specificity of chemical composition

Since it was observed that the *in vitro* toxicity is strongly product-dependent (2.2.3), it was speculated that this product specificity also applies to the chemical composition. Indeed, the number of chemical features detected in one product strongly differed between the samples. Some trends seem to exist in such that PVC, cellulose- and starch-based materials contain a higher and PET products a lower variety of features than other plastic types (A1–A3). The latter observation has also been reported for other PET products (Qian et al., 2018). However, the picture is characterized by exceptions that prevent making a generalized statement for all products of the same material type. Additionally, most compounds are unique to one product. For instance, half of the chemical features detected in one PLA product were absent in the

other PLA products, or, as another example, less than 1.1% of all features detected in one bioplastic or plant-based material were present in all samples of that material (A2). This low similarity of samples within one material type is also reflected in differences in total peak areas (A1) as well as in full-mass spectral data that comprises the abundance of all features (A1, A2). This shows that products of one polymer type do not share a chemical signature.

Likewise, the number of chemicals contained in plastics that is also leachable in water is product-specific. While nearly 50% of chemicals in analyzed PUR products were readily leachable, as an exception, one PUR product leached only 17%. The reverse was true for PLA; here, three out of four PLA products released a low number of compounds while from one PLA more than 5000 chemical features were readily leachable. As a proxy for quantity, the abundances of a chemical feature in methanolic extracts and aqueous migrates were compared. This indicates that the majority of compounds do not leach into water to their full extent. Still, many compounds were readily leachable since detected in comparable levels in corresponding migrates and extracts (A3).

It becomes clear that even if sharing the same polymer backbone, materials differ on their structural and chemical levels which results in a diverging degree of chemical release. Expressed positively, no matter the polymer type, careful product design can reduce the migration of compounds (see 2.6.4). To make sure that a product does not leach its inherent chemicals, migration tests need to cover all conditions a product may encounter during or after use. The reason for this is that several factors influence leaching such as the properties of the packaged good and physico-chemical drivers (e.g., contact time, temperature and area) as well as the thickness of the material (Bradley et al., 2019).

2.2.7 Identity, functionality and toxicity of plastic chemicals

The comparison of chemical features with several databases for their tentative identification showed that extractable and leachable chemicals comprise monomers and additives (e.g., plasticizers, antioxidants, flame retardants and UV filters), solvents, lubricants, process regulators and intermediates, as well as NIAS (A1–A3). Besides these plastic-associated compounds, chemicals associated with the packaged content were also identified. Of the identified compounds that were detected in more than one sample, only few appeared to be specific for a certain polymer type, for example, benzene (CAS 71-43-2) and styrene (100-42-5) which were only found in PS extracts (A1) or squalene (111-02-4) in PP migrates (Qian et al., 2018). The remaining chemicals were present across different material types (A1, A2).

Tentatively identified compounds detected most commonly in conventional plastics included the antioxidants butylated hydroxytoluene (CAS 128-37-0, 7 detects) and Irganox 1300 (6386-38-5, 6), the emulsifier methyl isostearate (5129-61-3, 6), 1,7-di-iso-propylnaphthalene (–, 6) used as plasticizer or solvent, as well as multiple cyclic compounds and ethylene glycols (A1, A3). The most prevalent chemical features present in bio-based and biodegradable materials were probably PLA oligomers (found in up to 35 samples; A2). PLA oligomers are commonly detected in PLA leachates; the type of oligomer depending on the applied extraction method (Ubeda et al., 2019a). Other notable compounds found in bio-based and biodegradable materials and previously associated with plastics comprise the antioxidant Irganox 1076 (CAS 2082-79-3), the additive erucamide (112-84-5) and the NIAS tetraoxacyclotetracosane-tetrone (141850-18-2; A2; Sage et al., 2018; Tian et al., 2020; Vera et al., 2018). Interestingly, the tentatively identified compounds of the present thesis did not comprise chemicals that are typically associated with plastics such as BPA and phthalates. This points out that the majority of plastic chemicals, including their potential hazards, are poorly characterized.

NIAS are another important group that needs consideration in the analysis of plastic chemicals. Over the last years, awareness for these substances has been increased but there is still a lot of research and regulatory advancement needed to better take into account their potential health hazards (Geueke, 2018). One challenge is that NIAS identification is particularly difficult (discussed elsewhere: Nerin et al., 2013). In the conventional plastics analyzed in the present work, seven NIAS were detected among the 260 tentatively identified compounds (A1). Other recent studies that performed high resolution non-target chemical analysis on plastic FCMs, have also detected different numbers of NIAS among identified chemicals. Bauer et al. (2019) identified 42 compounds including 8 NIAS, Gómez Ramos et al. (2019) identified 23 NIAS out of a total of 26 chemicals, while Vera et al. (2018) found that 57 out of 76 compounds were NIAS. Likewise, Carrero-Carralero et al. (2019) reported that the majority of the 107 compounds were NIAS, while García Ibarra et al. (2019) also discovered NIAS as being among their 100 identified compounds. This illustrates that NIAS can make up a remarkable part of the chemicals in plastic FCMs and that they need more consideration in safety assessments. However, the evaluation of their hazards is particularly challenging since toxicity data for most NIAS is missing (Geueke, 2018).

Information on potential hazards of plastic-associated chemicals is generally lacking or not publicly available (Groh et al., 2020). Only for 23% of the tentatively identified chemicals in conventional plastics of the present study was *in vitro* data on cytotoxicity, oxidative stress,

antiandrogenicity or estrogenicity available on ToxCast (A1; United States Environmental Protection Agency, 2018). These compounds included 21 chemicals that were prioritized based on their high toxicity and abundance, such as the well-known additives benzophenone (CAS 119-61-9), butylated hydroxytoluene (128-37-0) and triethyl phosphate (78-40-0; A1). It is unlikely that the few available toxicity data will explain the overall toxicity of a product. Although mixture toxicity concepts, such as concentration addition, are able to predict the combined effect of multiple chemicals for most classes, new evidence for synergism exists, especially for EDCs (Martin et al., 2020). This again emphasizes the great potential of whole migrate testing to contribute to hazard assessments. Here, toxicities of all compounds (previously described or not), as well as interactions of chemicals, are considered.

In the present work, the peak areas of all masses detected in a sample were taken as a marker for complexity. This complexity did neither allow predicting a sample's toxicity. Indeed, there was a trend of an increased toxicity with a higher chemical complexity but, still, there were samples with a high chemical complexity but a low toxicity and vice versa (A1, A2).

2.2.8 Unknowns and challenges in compound identification

During the tentative identification of plastic chemicals it became clear that most compounds remain unknown. While 82% of the chemical features in the extracts of conventional plastics could not be identified (A1), it were 6% for the extracts of bioplastics and plant-based materials (A2). Of the chemical features detected in the combination of migrates and extracts, 97% remained unidentified (A3). This illustrates not only that the majority of plastic chemicals is unknown but also draws the attention to challenges of technical and methodological nature: It is difficult (if not infeasible) to compare data generated by chromatography coupled to mass spectrometry if not proceeding in exactly the same manner.

In this context, in the studies of the present thesis different instruments and data analysis techniques were applied which led to a divergent identification success. For instance, in the first study GC-QTOF-MS/MS was used and mass spectra were identified using the NIST 14 library (score $\geq$ 70; A1). In the case of the second study, LC instead of GC was performed and only selected chemical features were identified by a combination of *in silico* fragmentation and ChemSpider search (match factor > 50; A2). In the third study also LC was used but, upon *in silico* fragmentation, three specific databases were queried for all detected chemical features (score > 40; A3). Putting aside the differences in the procedures, others that

performed GC-QTOF-MS analysis of PP food containers found 18% of compounds to be unknown (Carrero-Carralero et al., 2019), whilst the LC-QTOF-MS/MS analysis of plastic- and glass-related chemicals in honey revealed that more than 99% of compounds remained unidentified (Eyken et al., 2020). This shows that unknowns in plastics definitively need to be considered in hazard assessments. Unfortunately, the variability between studies prevents evaluating the exact extent to which the chemical composition of plastics is known/unknown.

A general reason that studies are of low comparability is that standardized and harmonized methods are lacking. This results in the use of different techniques, e.g., in the extraction of chemicals or the analysis of data. Furthermore, chemical databases are limited in their coverage of chemicals used in the manufacturing of (semi)synthetic polymers, and analytical standards are missing (Martínez-Bueno et al., 2019). As a consequence, the untargeted approach is susceptible to low and incorrect identification, respectively. In addition, some of the tentatively identified compounds in the present thesis had a very low match score. This illustrates the urgent need to improve and standardize workflows and databases to handle these thousands of chemical features and to reduce the unknowns. One important step is the recently published suspect list of chemicals used in FCMs, including plastics, that covers 12,285 IAS (Groh et al., 2020). In addition, effect-directed analysis (EDA) can help to prioritize compounds with potential adverse health effects for a further in-depths analysis. In EDA chemical and toxicological techniques are used in an interactive mode to identify the compound causing toxicity while also considering the unknowns (Vinggaard et al., 2020).

Taken together, plastics are characterized by known compounds with a known or an unknown toxicity, known compounds with an unknown presence in plastics (since not the typical suspects), known unknowns, as well as truly unknowns. Consequently, the majority of plastic chemicals are not only of unknown hazard but also unregulated.

2.2.9 Lacks in transparency

Complementary to the named unknowns, actors along the product life cycle face further unknowns since they lack information. The inter- and transdisciplinary cooperation within the PlastX project allowed to expand the findings made during this thesis by the findings of a social scientist and a practitioner (A5). This revealed these further unknowns. Overall, the study resulting of that cooperation shed light into ‘intransparencies’ (e.g., plastic ingredients are ‘intransparent’ since unknown) at distinct levels and points of the plastic life cycle of the

actual ‘transparent’ material (e.g., see-through). Subsequently, recommendations on how to achieve a fully ‘transparent’ packaging were derived. Aspects relevant for this thesis are that manufacturers of plastic pellets and products do not enclose information on known ingredients since they fear to give up proprietary information to competitors. Concurrently, often little effort is invested in communication throughout the product life cycle. Therefore, even the plastic producers may not know the exact composition of their product. This means that not only the NIAS and further unknown compounds but also the additives, which are known to the producer adding them, are kept concealed to others. This hinders the development of safe materials. In the further course of the plastic life cycle, this prevents product manufacturers and retailers to live up to their claim of offering sustainable and safe products. All these missing transparencies create additional, but avoidable, unknowns that impede production, choice and sale of chemically safe products.

2.2.10 Conclusion

Everyday plastic consumption brings about that humans are exposed to plastic chemicals. These chemical mixtures are characterized by a highly complex and diverse composition, as well as unknown compounds, and can induce *in vitro* toxicity. This raises concerns on the safety of plastics currently in use. Accordingly, future research is essential that reflects realistic conditions, and, thus, allows evaluating whether these concerns are justified. Furthermore, the classical approach to test and assess the toxicity chemical by chemical is time consuming and not sufficiently informative. Therefore, new scientific approaches and regulatory measures acknowledging the chemical complexity and diversity of plastics are imperative to improve their chemical safety (see 2.6).

2.3 Bioplastics – A regrettable substitution?

With the increasing public awareness for the downsides of plastics and demands for eco-friendly alternatives, the pressure on the plastic industry is rising to meet this demand. Bio-based and biodegradable materials only account for 1% of today’s plastic market but are currently the prevailing solution option (European Bioplastics, 2019). The prefix ‘bio’ and their marketing as sustainable alternatives suggest that these materials are free of any negative impact on human and environmental health. However, do they ‘keep their word’ or is it a fallacy and are they a regrettable substitution?

2.3.1 Safety aspects

As indictor for the safety of a material, in the present work the toxicity of the chemical mixture contained in the material was analyzed with *in vitro* bioassays. This illustrates that bio-based and biodegradable materials do not outcompete conventional plastics but are similarly toxic. Along that line, the comparison of extractable chemicals for unspecific and endocrine *in vitro* toxicities shows that both classes share the same prevalence of toxicity (67% of the products) and have comparable mean effect strengths (A2). Thus, changing from 'conventional' to 'bio' does not necessarily imply a higher safety. The few studies that have investigated effects of 'biomaterials' support the notion regarding their toxicity. Correspondingly, chemicals and micro-sized particles of bio-based and biodegradable materials adversely affected marine (Magara et al., 2019) and freshwater organisms (A4; Göttermann et al., 2015), terrestrial plants (Serrano-Ruíz et al., 2018) and whole vegetation (Menicagli et al., 2019), as well as soil quality (Accinelli et al., 2020) and bacteria (Adhikari et al., 2016). In direct comparisons of conventional plastics and their alternatives, PLA was proven to be as toxic as petroleum-based plastics (A4; Green et al., 2016). For instance, PLA microplastics had a similar effect on reproduction as PUR microplastics and induced an even higher mortality as PUR and PVC microplastics in *D. magna* (2.4; A4).

Just as conventional plastics, bio-based and biodegradable materials can contain a high number of compounds. Accordingly, up to 20,000 chemical features were detected in an individual bioplastic sample (A2). While reports on the total number of chemical features detected in bio-based and biodegradable materials are still absent, some studies have stated the number of identified chemicals. Ubeda et al. (2019b) identified 15 volatile compounds in a PLA-PE-blend while Bradley et al. (2010) reported the identity of 32 and 29 volatile and non-volatile compounds, respectively, in 13 migrates from bio-based FCMs. A further study identified 67 volatile compounds released by biodegradable materials, including chemicals common to conventional plastics (Asensio et al., 2020). The usage of the same chemicals in conventional and bioplastics appears plausible since both require additives to achieve the desired properties and performance. In the case of materials such as PE, a change in the carbon source of the monomer from petroleum (PE) to renewable feedstocks (Bio-PE) does not imply a change in further processing. Correspondingly, petroleum- and bio-based PE products shared a similar *in vitro* toxicity (A2). On the other hand, some chemicals are rather present in bioplastics, simply due to the production of the natural resources they are made of (e.g. pesticides and fertilizers; Gironi and Piemonte, 2011a). Furthermore, bio-based FCMs

have been linked to be a source of NIAS, such as acrylamide and dioxins that result of their processing (Bonwick et al., 2019).

The material a bio-based/biodegradable product is made of allows neither predictions on its toxicity nor its chemical composition (see 2.2). However, the cellulose- and starch-based products analyzed in this thesis, overall, contained above-average numbers of compounds and induced high levels of toxicity (Table 1; A2). Especially polymers extracted from natural resources, such as starch and cellulose, but also other bio-based polymers that are decomposable in nature, have inherent limitations such as brittleness, a poor moister and gas barrier, as well as processability. As a result, to make replacement of conventional plastics possible, these bio-based polymers are blended with further (petroleum-based) polymers and modified with fillers, plasticizers and other additives which increases their chemical complexity (Khan et al., 2017; Mekonnen et al., 2013; Scarfato et al., 2015). Thereby, the feedstock used to produce a material strongly influences the product's elemental composition and the migration of contaminants (Astolfi et al., 2020).

2.3.2 Other sustainability aspects

When evaluating a material's sustainability, in addition to safety, further environmental impacts need consideration. One evaluation option, e.g., implemented in LCAs, is to assess the overall ecological footprint of a product taking the whole product life cycle into account. Concerning the feedstock source, bio-based materials have the big advantage that they conserve fossil fuels. Thus, according to LCAs, they can be superior to conventional plastics in resource consumption as well as in greenhouse gas emission. Still, bio-based materials can, ultimately, have a higher impact on ecosystem quality and human health. Factors contributing to theses impacts are that their production is associated with the consumption of land and water, the use of pesticides and cultivation in monocultures (Federal Environment Agency, 2012; Gironi and Piemonte, 2011b). At the same time, crop production for bio-based materials may have societal impacts, e.g., if land use competes with food production. These factors can be bypassed if using sustainable agriculture methods and residues of food production as the starting material (Álvarez-Chávez et al., 2012). Generally, it is difficult to identify the most sustainable product based on LCAs since the comparability of different LCAs is limited. On the one hand, the described material complexity and diversity of plastics and their alternatives impedes not only (eco)toxicological risk assessment but also LCAs. On the other hand, the variety of applied processing methods and distribution steps a product

passes through make LCAs even more complicated. Eventually, the outcome of a LCA depends on the analyzed item, its production and distribution, as well as on the type and weight of criteria applied in the assessment (Giovannini et al., 2015). Moreover, at the moment LCAs leave aside toxicological aspects associated with the use of products. Instead, they only consider exposure to chemicals by environmental emission, for instance, due to feedstock cultivation (Ernstoff et al., 2019).

With regard to environmental impacts at the end-of-life and aesthetical aspects, biodegradables are a promising solution for solving the world's plastic litter problem since they break down to CO_2, water, biomass and inorganic salts. However, biodegradation largely depends on the environmental conditions, such as temperature, humidity and the presence of microorganisms. As an example, PLA may decompose in industrial composting facilities within days or months. In aquatic environments, this would take years, if happening at all, due to low temperatures and the limited concentration of microorganisms (Haider et al., 2019). This phenomenon is not exclusive to PLA since other biodegradable polymers have neither degraded in sea- nor freshwater within a year (Bagheri et al., 2017). This points to the fact that the requirements on a product during use (e.g., carrying water) compared to after-use (e.g., disappearing in water), are mutually exclusive (Lambert and Wagner, 2017). The development of a one-size-fits-all solution, such as biodegradation at the touch of a button, seems unlikely. Therefore, biodegradables need clear labeling to point out the correct disposal route so that consumers are not left confused. Confusion also exists regarding the differences between 'compostable' and 'biodegradable'. EU-wide standardized comprehensive definitions and certifications of the two terms would contribute resolving these confusions (IG Plastics, 2018). Due to the above-mentioned potential toxicity on wildlife, certification should include further ecotoxicity measures besides plant germination and growth which are currently considered by the European standard EN 14432 (DIN, 2000). Others aspect are that criticism on the composting of biodegradable plastics has been raised as they can disrupt the functioning of industrial composting facilities (German Environmental Aid, 2018) and that several reports state that the process of composting itself is ecologically inferior to thermal recycling (Federal Environment Agency, 2012). Besides, biodegradable materials are mostly intended for single-use which conflicts with a circular economy (see 2.6.4). Taken together this suggests that, for the moment, biodegradables are appropriate for niches. This means for applications where degradability is part of the function (e.g., as a carrier of active ingredients in medicine), or where collection is unlikely (e.g., cartridges of fireworks), or, if recycling is

not practicable, (e.g., agricultural film due to dirt), or if environmental entry is inevitable (e.g., fishing nets; IG Plastics, 2018).

2.3.3 Conclusion

At present, bioplastics and plant-based materials can outperform conventional plastics regarding their environmental impact, for instance, if their feedstock cultivation and processing is environmentally sound or if biodegradability is advantageous. Despite this, they seem not yet the solution with regard to safety aspects (see 2.6 for improvement options). Together with their high potential of environmental release and scarcity of studies on materials other than PLA, this stresses the necessity for a thorough toxicity investigation of bio-based and biodegradable materials. Here, potential implications for human health should gain more attention, since packaging is the main market segment of bioplastics (European Bioplastics, 2019) and ambiguity exists whether they meet the legal requirements for food contact (Scarfato et al., 2015). One great advantage of 'biomaterials' over conventional plastics is that they have a positive connotation in society: this can support their growth, despite having a higher market price as conventional plastics (Federal Environment Agency, 2012). Consequently, the bioplastic industry should handle that trust with care and implement what the name suggests: sustainability on every level, not only with regard to environmental but also with regard to human health aspects.

2.4 Drivers of microplastic toxicity to *Daphnia magna*

While it is well studied that microplastics can negatively affect aquatic organisms, such as *D. magna* (e.g., Schür et al., 2020), investigation on the factors responsible for these effects are scarce. Keeping the chemical heterogeneity and product-specific toxicity of plastics in mind, in the present work, the toxicity of microplastics made of currently understudied polymer types were compared in chronic exposures of *D. magna*. Subsequently, the role that plastic chemicals play in microplastic toxicity was analyzed (A4). Therefore, irregular microplastics (≤ 59 µm) were produced from three products whose chemicals induced high baseline toxicity *in vitro* (Table 1; A1)[15] and that are based on PVC (corresponds to PVC 4 of A1), PUR (PUR 1) and PLA (PLA 3). Since the aim at that stage was to investigate the mechanisms of toxicity

[15]Baseline toxicity generally correlates well with toxicity in *D. magna* (Kaiser, 1998).

and not environmental risks, higher concentration than currently occurring in freshwaters, but likely to cause an effect, were applied in the first experiment (A4).

2.4.1 Chronic effects of microplastics

The results illustrate that PVC, PUR and PLA microplastics can have negative effects on freshwater invertebrates. Accordingly, all three microplastic types affected life-history traits of *D. magna* with a higher efficiency than the natural particle kaolin (A4).

The effects seem to be material-specific. While PVC was most toxic to reproduction when comparing mass (EC_{50} = 45.5 mg L^{-1}, Table 1) as well as numerical particle concentrations (EC_{50} = 1.14 × 10^7 particles L^{-1}), PLA induced the highest mortality (60% at 500 mg L^{-1}; A4). To date, other microplastic toxicity data on PUR is absent and few studies have examined and compared the impact of PVC and PLA on *D. magna*. Renzi et al. (2019) reported that microplastics based on PVC had a lower acute toxicity than PE and PP microplastics. Schrank et al. (2019) compared rigid and flexible PVC. The authors observed that rigid PVC delayed primiparity and flexible PVC altered the body length and reproductive output under fasting conditions. These studies underline the results of the present thesis that PVC can have negative impacts on *D. magna.* At the same time, the latter study emphasizes that toxicity is not solely dependent on the polymer type but rather depends on the chemical composition of a (micro)plastic. Similarly, the PLA microplastics used in the present thesis affected the survival, reproduction and body length at a high concentration (500 mg L^{-1}; A4), while other PLA microplastics did not influence feeding, size and population growth (Gerdes et al., 2018). However, in the study by Gerdes et al,, the microplastics were only of one size (2.4 µm) and of lower concentration (19.6 µg L^{-1}) which may also explain the differences. Generally, micro-sized bioplastics, such as PLAs or PHAs, can cause similar effects as conventional plastics in *D. magna* (A4) as well as other aquatic organisms, such as *Gammarus fossarum*, *Ostrea edulis* and *Arenicola marina* (reviewed in Shruti and Kutralam-Muniasamy, 2019).

Altogether this highlights that research needs to reflect the diverse picture of polymers and chemical ingredients that actually occur in the environment instead of focusing on individual polymer types and microplastic without chemicals. Only then, a realistic hazard characterization of microplastics becomes possible.

2.4.2 Differentiation between chemical and physical toxicity

To clarify whether plastic chemicals rather than the particle itself are responsible for microplastic toxicity, microplastics as such were compared to particles without extractable chemicals, chemicals extracted from microplastics with methanol and chemicals that leach from microplastics into water. The results demonstrate that plastic chemicals or the particle itself can be the main driver for toxicity. This depends on the microplastic type. In the case of PVC, the chemicals contained in the material reduced the size and reproduction of *D. magna* and delayed the day of the first brood, whereas, in the cases of PUR and PLA, microplastics with and without chemicals affected several life-history traits of *D. magna*. For PUR and PLA, the mere particle was at least as toxic as the microplastic with chemicals (A4). This implies that the physical properties of the fragments caused the toxicity. For again other microplastics, toxicity can result of a combination of chemical and physical properties. A study with PE fragments reported a synergistic acute toxicity of the particle and the additive benzophenone-3 contained in the microplastic on *D. magna* (Na et al., 2021). Another study identified particle-related, (e.g., disruption of the cell membrane by sharp edges) as well as chemical-related effects (e.g., cell damage due to reactive oxygen species (ROS) production) as contributing factors to the overall *in vitro* toxicity on cells (Choi et al., 2020). Contrary to this, chemicals were responsible for the toxicity of polycarbonate (PC) granules on a garden cress (Pflugmacher et al., 2020). In line with the findings of the present thesis, Oliviero et al. (2019) attributed toxic effect of PVC microplastics on sea urchin to the leachable chemicals. Studies on other polymer types observed that chemical leachates neither caused the effect of PET fibers on the survival of *D. magna* (Jemec et al., 2016) nor the effect of PS beads on the reproduction of *C. elegans* (Mueller et al., 2020).

All in all, chemicals can be the main driver of microplastic toxicity, as in the case of the PVC studied here. However, this depends on the respective microplastic type. Accordingly, for other microplastics, such as for the analyzed PUR and PLA, the physical properties of microplastics determine the effects (A4) and for yet others it can be the combination of both chemical and physical characteristics.

2.4.3 Chemical toxicity

In order to find an explanation for the different toxicities of the PVC, PUR and PLA extracts investigated in the present thesis, their chemical profiles were compared. Neither the variety (peak number) nor the abundance (total peak area) of chemicals and not the presence of

priority chemicals (according to abundance and *in vitro* toxicity) could explain the observed differences (A1, A4). For instance, the PLA extract contained a higher number of chemicals than the PVC extract but was less toxic (A1). It remains to be clarified which chemical or combination of chemicals in the PVC extract caused the observed effects. Yet it is certain that toxic chemicals did not leach into water since the aqueous PVC migrate had no adverse impact on *D. magna* (A4). However, another aqueous migrate of the same material inhibited the bioluminescence of *Aliivibrio fischeri* in the Microtox assay (A3). Several factors may explain these differences in toxicity. For example, the twice as high temperature applied to produce the migrate for *in vitro* (A3) compared to *in vivo* (A4) testing, might have favored the release of toxic chemicals. Furthermore, the Microtox assay might be susceptible to more or other compounds, or has a higher sensitivity compared to *D. magna*. In contrast, PP and PS plastic pellets, leached for 24 h at room temperature in water, released compounds that induced not only baseline toxicity but also affected survival and reproduction of *D. magna*. Interestingly, these pellets were supposedly free of additives and monomers but still leached toxicity (Schiavo et al., 2018).

The literature provides clear evidence that plastic leachates adversely affect also other aquatic biota besides *D. magna* (Lithner et al., 2012; Schiavo et al., 2018; Xu et al., 2020), such as *Nitocra spinipes* (Bejgarn et al., 2015), *Amphibalanus amphitrite* (Li et al., 2016), *Potamopyrgus antipodarum* (Wagner and Oehlmann, 2009), *Littorina littorea* (Seuront, 2018), *Mytilus galloprovincialis* (Capolupo et al., 2020) and *Paracentrotus lividus* (Oliviero et al., 2019). Generally, these data confirm the above discussed that different species have a different susceptibility to plastic chemicals (e.g., Schiavo et al., 2018; Seuront et al., 2021) and that toxicity depends on the individual chemical composition of a (micro)plastic (see also 2.2). For instance, only leachates of some, but not all, PVC toys influenced larval development of the sea urchin *Paracentrotus lividus* (Oliviero et al., 2019). Likewise, the PUR analyzed here did not leach chemicals toxic to *D. magna* while other PUR products did (Lithner et al., 2009). When comparing results of different studies, it is important to consider that several factors influence the migration of (toxic) chemicals. Besides the characteristics of the material (e.g., crystallinity, structure, surface area) and properties of inherent chemicals, these further include the ambient conditions (e.g., salinity and turbulence) as well as the presence of microbes (Bradley et al., 2019).

Exposure of organisms to plastic chemicals happens not only via the ambient medium after they have been released from plastics but also via ingested microplastics when chemicals migrate during their residence in the gastrointestinal tract. Since data on the bioavailable

fraction of additives in ingested microplastics is missing, Fauser et al. (2020) estimated 1–10% to calculate the risk upon microplastic ingestion by marine species. The authors concluded that the leached additives can pose a risk but in a chemical- and species-dependent manner. An additional exposure route to plastic chemicals is through the ingestion of contaminated prey (Rochman, 2016b). These different routes, through which aquatic organisms can take up plastic chemicals, complicate the calculation of concentrations organisms are actual exposed to in natural environments. Moreover, wildlife is not only exposed to chemicals from plastics but also to chemicals from other sources. Data on the relative contribution of plastics to the overall exposure of wildlife to hazardous chemicals is conflicting. This suggests that the bioaccumulation of plastic chemicals in animals depends on several factors, such as physical and chemical properties of the chemical, concentration of exposure, ecology of the animal as well as the environmental matrix (Rochman, 2016b). In addition, other non-chemical stressors, such as food limitation, can enhance the toxicity of chemicals. Hence, all these stressors need to be considered when estimating safe exposure levels of plastic chemicals (Heye, 2019).

2.4.4 Physical toxicity

Apart from the chemical component, the physical characteristics of microplastic particles can determine their toxicity. Before discussing physical properties, it is noteworthy that in the microplastic experiments of the present work, a higher particle number did not mean a higher toxicity. For instance, 35-times more particles of kaolin were required to reduce reproduction to the same level as the least toxic microplastic type (Table 1, A4). Thus, other properties, than mere particle numbers, influenced toxicity.

One particle characteristic that can affect microplastic toxicity is size which was reported by several *in vivo* and *in vitro* studies (e.g., Jeong et al., 2016; Lee et al., 2013). Joeng et al. (2016) observed an increased toxicity when particle size decreases. This is well explainable since with the decreasing size the reactive surface, the interaction potential with cells and the tissue translocation increases (Stock et al., 2021) which again leads to an increase in toxicity, e.g., due to inflammation (e.g., Jeong et al., 2016). For several species, tissue and cellular translocation is most likely when particles are < 3 µm (Shang et al., 2014; Triebskorn et al., 2019). Contrary to other studies, in the present thesis, smaller microplastics did not induce higher effects (A4). Instead, PLA and PUR particles, mostly < 40 µm, triggered higher toxicity than PVC particles, mostly < 20 µm. However, due to technical limitations, the

quantities of particles < 2 µm are unknown. Thus, PLA and PUR particle exposure may have contained a larger fraction of particles < 2 µm responsible for the effect.

Besides size, particle shape can influence microplastic effects. According to several studies, microplastic fragments and fibers generally have a higher toxic potential than spherical particles (e.g., Au et al., 2015; Ogonowski et al., 2016). Potential explanations include that the former have a longer retention time in the digestive tract and that their sharp edges cause injuries (Lambert and Wagner, 2018). Since fragments and fibers are also predominant in the environment (Burns and Boxall, 2018), in the experiments of the present thesis only irregular microplastics with rough surfaces were used. While the PVC and PUR particles, that shared a similar rounded shape, did not share a similar toxicity, those with more dissimilar shapes did (A4). This suggests that shape was neither the responsible factor for the observed differences in the present work. Nevertheless, other shapes may play a more decisive role in microplastic toxicity which needs to be evaluated in further research.

Consequently, for the microplastics in this thesis, neither size, shape nor surface morphology appears to be the sole relevant factor for physical toxicity. Other particle properties that can affect toxicity and may have driven the effects of the PUR and PLA particles include density, crystallinity and surface charge. These factors can influence particle concentration (e.g., agglomeration reduces particle number), availability (e.g., density determines location in the water column) and interaction potential (e.g., surface charge influences the interaction with cells; Lambert et al., 2017; Lambert and Wagner, 2018). Environmental conditions (e.g., if favouring biofilm formation) as well as ageing, (e.g., leading to fragmentation and degradation of microplastics) will again change the particle properties (Lambert et al., 2017; Sørensen et al., 2021). This demonstrates that several, continuously changing physical characteristics are combined in microplastics and can influence their toxicity to a greater or lesser extent.

2.4.5 Conclusion

Microplastics are no homogeneous entity but a versatile combination of multiple plastic types, chemical ingredients and physical properties, all of which need to be considered when assessing and reporting their hazards and risks. However, it seems infeasible to individually evaluate every microplastic type occurring in the natural environment. In the present work, an alternative approach was chosen and tested on three microplastic types. Here, the idea is to identify the decisive factors that drive microplastic toxicity and to prioritize these in future

testing. The fact that in the present work PVC induced negative effects at lower concentrations than PUR and PLA and that chemicals were the responsible factor, serves as first indication that chemicals might play a more important role than particle properties in microplastic toxicity (A4). A recent study supports this assumption. Jeong and Choi (2020) analyzed the toxicity mechanism of the 50 most common plastic chemicals to identify the most relevant adverse outcome pathways (AOPs) of microplastics. The authors proposed neurotoxicity, inflammation, lipid metabolism and cancer pathways as the most relevant AOPs. These coincide exactly with the main toxicity mechanism attributed to microplastics. This certainly demonstrates that chemicals can no longer be neglected in the evaluation of microplastic effects but need prioritization.

2.5 Risk framing of (micro)plastics

2.5.1 Knowledge gaps on microplastics

In association with the complexity of microplastics, even after approximately a decade of intensive research, knowledge gaps still remain. Generally, the discrepancy between the microplastics studied in laboratories and those occurring in aquatic environments (e.g., regarding concentrations, sizes and plastic types) has to be addressed (Burns and Boxall, 2018). For a realistic exposure estimate, it is essential to solve the methodological limitations and to quantify microplastics < 50 µm (Gouin et al., 2019). Further knowledge gaps include, but are not limited to, the effects of plastic-associated chemicals (2.4), tire particles since they account for a large proportion of microplastics (Bertling et al., 2018) and nanoplastics since their small size makes tissue translocation possible (Wright et al., 2013). Including controls with natural particles is necessary to clarify that the effects observed are microplastic-specific (A4). A shift of research focus from the (sub)organismal to the ecological level is proposed and would be covered by multigenerational studies and multi-stressor exposures (Rochman, 2016a). Due to these knowledge gaps, many uncertainties remain that impede the prediction of microplastic risks. At the same time, closing these gaps is time consuming, postpones action and takes resources away from preventative measures.

2.5.2 Traditional risk framing and dissents

Notwithstanding the mentioned lacks of knowledge, Science Advice for Policy of European Academies (SAPEA) reviewed the existing literature to derive a statement on the ecological risks of microplastics for current environmental concentrations following the classical ecotoxicological approach. The report concludes that, even now, microplastics pose a risk (PEC > PNEC) at some locations and that risks may be widespread within a century if microplastic emissions remain the same (SAPEA, 2019). An alternative assessment by Koelmans et al. (2020) came to a similar conclusion. The authors developed a toolset that corrects for the diversity of microplastics and, thus, permits calculating the risk of one single material. The application of the toolset on several species sensitivity distributions (SSDs) of freshwater organisms demonstrates that in 1.5% of surface waters worldwide, for which the microplastic abundance is known, threshold effect concentrations are exceeded.

But which areas must be ‘at risk’ in order to take global action? This question is currently bypassed by many ecotoxicologists. They argue that existing data is too scarce for a sound evidence-based risk assessment and, thus, a risk decision. However, there is no universally agreed upon risk conception. Accordingly, aside from ecotoxicology, other disciplines apply other frameworks. Some have already classified microplastics as posing a risk, for instance, marine biology. Here, the criteria for risk are ubiquity, persistency and ingestion by biota, all of which are fulfilled by microplastics (Backhaus and Wagner, 2020). One may also argue that evidenced-based risk calculations, as performed by SAPEA (2019) and Koelmans et al. (2020), deliver sufficient information to designate microplastics as a candidate for the precautionary principle which applies in the EU. Correspondingly, potential harm for humans and the environment shall be prevented despite existing knowledge gaps (United Nations Environment Programme, 1992). Another scientific approach also suggests that plastics fall under that premise since they present a planetary boundary threat; plastics cause irreversible exposure on the global scale and have a currently not fully known disruptive effect on a vital process of the Earth system (Jahnke et al., 2017). This shows that depending on the conceptualization, microplastics are or are not classified as risk.

One the one hand, different scientific disciplines follow different concepts. One the other hand, scientists themselves adopt different perspectives on the topic, driven by their personal values. Some scientists consider health effects at the individual level as important as those within an entire population. As a consequence, they argue that the existing evidence is sufficient to take measures (Liboiron, 2016). Others state that, despite the ample

demonstration of widespread contamination, there is little evidence for this contamination being the cause of any ecological harm (Rochman et al., 2016). Again, others rely on the precautionary principle. This absence of a consensus among scientists and with that also of a single judgment complicates the structuring of the problem. This, in turn, complicates policy decisions as the basis for holistic and effective measures (Shaxson, 2009). An additional compounding factor for systemic risks, such as plastic pollution, is that the creation of new knowledge will reveal more variables and, thus, make political interventions even less likely (Kramm and Völker, 2018). This all leads to the fact that systemic action to address the issue is still lagging behind.

2.5.3 Moving beyond traditional risk framing

It is also argued that traditional environmental risk calculation is generally inadequate to evaluate systemic risks such as plastic pollution. Here, the focus is on ecological impacts, while other impacts, such as the societal significance of plastic-associated risks, are neglected.

The social-ecological perspective, as adopted in PlastX, also accounts for these social dimensions. Societies consider more than technical or toxicological data and deliver many arguments for action on microplastics and plastic litter (Völker et al., 2017). From an aesthetic point of view, any piece of plastic in the environment is 'matter out of place';[16] it is an anomaly that reduces the feel-good factor. Individuals have different perceptions of harm and risk and, thus, may regard 'matter out of place' or microplastics as unacceptable (Liboiron, 2016). The aesthetical mismatch becomes an economic issue, for instance, when income is lost due to marine litter (Andrady et al., 2015; Krelling et al., 2017). A calculation on the loss of income from marine tourism, fishery and aquaculture, as well as the cost of clean-up activities, due to land-sourced plastic waste, estimated that for 87 coastal countries USD 6-19 billion were lost, with 82% due to clean-up activities (Deloitte, 2019). Furthermore, plastics, during and after use, might contradict people's moral concepts. For example, the waste of resources is incompatible with sustainability claims and the entanglement of wildlife with ethic principles (SAPEA, 2019). Moreover, consumers perceive microplastics as a threat for their health due to particles found in food (Federal Institute for Risk Assessment., 2016). One factor contributing to this perception is that the public conceptualizes something as risk based on the uncertainty of a negative outcome and not the negative outcome itself. In addition, the media neglect the communication of uncertainties

[16] "*Where what is in and out of place is [, itself,] determined by religious norms, practices and values*" (Liboiron, 2016).

which turns scientific hypotheses into facts (Völker et al., 2019). As a result of the perceived threat, many consumers seem to desire change. Moreover, other actors in the plastic system, such as product manufacturers, have expressed that measures should be taken (A5). As described above, scientific experts adopt different positions on the topic that is surrounded by uncertainty. While some recommend action, others do not. In the interim, societies have already started to take action neglecting that science is not yet ready (Kramm et al., 2018). This contradicts the traditional procedure in which a data-driven risk identification by scientists forms the basis for risk management by societies.

2.5.4 Conclusion

The ecological and societal complexity and interconnectedness of plastics-associated problems contradicts a classical quantitative risk analysis. This approach appears to be insufficiently protective for plastics and other global environmental issues of the Anthropocene (Kramm et al., 2018). Consequently, new approaches have become necessary to assess and manage these issues. The adoption of a social-ecological risk perspective combined with an inter- and transdisciplinary research approach, as applied in the PlastX project, represents a promising way forward. The integration of experimental findings of this thesis with other research outcomes of PlastX allowed to identify constraints for change, as well as to propose applicable and sustainable solution options as foundation for problem solving.[17]

2.6 Ways forward to a better plastic economy

Many problems accompanying plastic use are somehow connected with plastic waste that is, simultaneously, also the most visible symptom of the problem. One pillar to tackle the world's waste problem is to increase waste management capacities. However, this alone cannot keep pace with the projected growth in plastic waste generation (Borrelle et al., 2020). In the context of plastics already in the environment, removal of 'matter out of place' is unsustainable, labor- and cost-intensive, or even impossible, especially in remote locations (e.g., deep sea) and when the size of litter decreases (SAPEA, 2019). Since more than 90% of

[17]The chapter 2.6 describes these findings. For a better classification, it might be important mentioning that parts of the described are personal observations made or discussed during the work in the PlastX project. In these cases no reference may be given. Additionally, it is not comprehensively accounted for every aspect since this would exceed the scope of the present thesis.

marine plastics is smaller 5 mm (based on particle count), it appears infeasible to clean it all up (Eriksen et al., 2014). Thus, to solve the problem of plastics in the environment, it is more effective, in the long-term, to focus on the sources instead of the sinks. At the same time, this allows tackling other downsides of plastics which may already be relevant during the production, distribution or use phase of the material such as safety aspects.

2.6.1 The past and the present – How we got to where we are

In order to identify the source of the problem, it is helpful to outline how we got to where we are now. With the beginning of the plastic age in the 1950s, this new material provided society with many benefits such as health protection due to sterile packaging, energy conservation when used in insulation, and new modes of transportation including aircrafts. Design for linearity brought convenience (e.g., less dish washing) and low pricing made it affordable at the same time. Society was trained for single-use and the material was given little value (Andrady et al., 2015).[18] In addition, from the waste perspective, this way of consumption was affordable since low production volumes permitted an easy management of the resulting waste. However, with the tremendous increase in plastic production and consumption, this has changed and plastic litter has become the symptom of an inefficient, outdated business model (Borrelle et al., 2020). From the social-ecological risk perspective, it is an unintended by-product of normal every-day life (Keil et al., 2008). This highlights that the material itself is not the problem. Instead, the problem lies in a combination of different interconnected factors, including design (for linearity), marketing (throw-away, little value) and consumer behavior (improper disposal). With regard to consumption, overall living conditions (e.g., poverty) may even rule out any deliberate choice-making, such as to renounce the use of plastics or to dispose them properly. This multifaceted nature of the issue is troublesome itself since it permits actors to deny their responsibilities and shift them to others when it comes to solving the issue. Furthermore, plastics are ubiquitous: today's markets depend on plastics and so do societies. This dependency, together with the ambivalence of the material, i.e., happiness and ruin at the same time, makes solving the

[18]The advertising slogan *"Plastic is forever … and a lot cheaper than diamonds."* illustrates the manner the material was marketed; as something affordable ("*cheap*") that brings happiness ("*diamonds*") and this for a long time ("*forever*"). One may even interpret that plastics are an easy way ("*cheap*") to bring happiness to the housewife ("*diamonds*"), since diamonds are stereotypically a gift to woman and "*plastic*" was marketed as a good that brings relieve to the housewife (e.g., no need of cleaning up the dishes, since it can just be thrown-away after use).

problem even more challenging. To address the multiple scales of the issue, no single solution will suffice and so many strategies are required (Kramm and Völker, 2018).

2.6.2 The future – The favorable plastic

It can be argued that the ultimate goal ('go-to') instead of the current situation ('as-is') should be kept in mind when aiming for an effective transformation of the plastic economy. The final goal is to achieve overall sustainability[19] and safety while retaining the advantages that plastics deliver. These advantages include easy production, cost-effectiveness and broad applicability. The latter is made possible by favorable characteristics such as transparency, durability, light weight and flexibility. At the same time, the whole product life cycle should be environmentally sound (e.g., low carbon footprint) and socially acceptable (e.g., not competing with food production for feedstock). In order to minimize resource consumption, the material should have a long service life and, afterwards, re-enter the product life cycle without high energy expenditure. Furthermore, it is desirable that it instantly and completely biodegrades upon entering natural environments, irrespective of the prevailing conditions. At all stages, the material should entail no negative impact on human, wildlife and overall ecosystem health. This implies that the material itself is free of toxic chemicals, as is its whole production process and handling.

2.6.3 Solutions – Parts of the puzzle

One step on the way to achieve this desired state of sustainability is to reduce both product and resource consumption wherever possible. This includes the elimination of non-essential uses such as double packaging. Another step towards reduction is the measures of the European Parliament to minimize the environmental impact of the ten most commonly littered single-use plastic items (EU, 2019). It is important to avoid rebound effects, when reducing plastic consumption. This means that resource gains, due to plastic reduction, should not entail alternative downsides (e.g., more food loss due to faster food degradation; Andrady et al., 2015). A third step is to reuse and repurpose products wherever possible to prolong the life of the resources in use. This includes platforms for the sharing and redistribution as well as the up-streaming of waste; an example is the recapture of fishing nets and their reuse for

[19]The modern use of the term 'sustainable development' follows the description of the 1987 Bruntdland Commission Report "Our common future" as "*development that meets the needs of the present without compromising the ability of future generations to meet their own needs*" (World Commission on Environment and Development, 1987).

the production of other valued items. Replacement is an option if there are alternatives that outperform plastics in all aspects. For instance, sugars can replace microbeads in cosmetics. Finally, if there are none such alternatives and plastic use brings benefits, the redesign and rethinking of plastics is essential.

2.6.4 Redesign and rethinking – Key factors for a desirable plastic economy

Two pivotal pillars that need consideration in the redesign and rethinking of plastics are impacts on human as well as on environmental health. Regulation is a key factor that promotes both.

As elucidated by the present thesis, it is not warranted that plastics are safe. For FCMs, this indicates that the EU regulation EC10/2011 for plastic FCMs is not sufficiently effective to ensure human health protection at its current state (A1–A3; EU, 2011a). Thus, a paradigm shift in the way potential health effects are unraveled is imperative. The experimental approach applied in this work serves as an example on how that shift can be supported. In contrast to the common approach to analyze the migration of starting substances, in the present thesis, whole migrate toxicity testing of the marketed products was performed. This considers the toxicity of unwanted and unknown side products of processing (e.g., NIAS) as well as mixtures effects (Muncke et al., 2017; Muncke et al., 2020). Moreover, regulation EC10/2011 only addresses genotoxicity and mutagenicity of plastics but, as demonstrated in the present work, further toxicological endpoints need consideration such as endocrine activities (A1–A3). The fact that compounds, such as EDCs, may have a non-monotonic dose-response relationship, additionally points out that a generic migration limit, as used in the current approach, is inappropriate to cover toxicity occurring below that limit (Muncke et al., 2017). Further proposed improvements of the plastic FCM regulation include the reassessment of authorized substances and their toxicity thresholds, the exclusive authorization of chemicals with available analytical standards and the addition of currently disregarded substance classes such as oligomers and printing inks (Muncke et al., 2017; Muncke et al., 2020). Moreover, there is the urgent need to harmonize EC10/2011 with overlapping European regulations such as REACH and national protocols of the member states. It is also necessary to facilitate and better assure the implementation of legal requirements. Therefore, it should be thought through how to guide companies in safety assessments and transparent reporting as well as how to survey compliance (Grob, 2019;

Pawlicka et al., 2020). Movement around this topic has already begun; advancements include an ongoing evaluation of the EU FCM legislation (EC) No 1935/2004 (EU, 2004) to assess whether it is fit for purpose and delivers as expected (EC, 2020a). Here, a recent report identified, among others, gaps due to the complexity of polymers and the migrating chemicals, as well as deficiencies in the consideration of NIAS. The report further states a lack of, but desire for, harmonization such as measures covering all FCMs in the form of a Union list or guidelines (EC, 2020b). Furthermore, in October 2020, the EC published its new Chemicals Strategy for Sustainability (CSS) in which it sets out strategies to enforce safe and sustainable chemicals. This also includes plans for the EU's FCM regulation such as the consideration of mixture effects as well as the omission of chemicals with reproductive or endocrine effects (EC, 2020c). It remains to be seen how these lines of action will progress.

The finding that every plastic(-like) product has an individual chemical composition and toxicity (2.2.3, 2.2.6) implies that each product has to be evaluated separately to ensure its safety. However, an individual assessment of each product, considering the number of polymers, chemicals and plastic products on the market, would entail extensive toxicological and analytical testing. This would be very expensive, time consuming and, thus, barely realizable. A reduction of complexity would facilitate testing and support the safety-by-design principle. This can be achieved by authorizing only a limited set of safe chemicals or materials via whitelists (A5). According to the results presented in this thesis, non-toxic products are already available on the market (2.2.3). Consequently, products can be manufactured in a way that they are free of toxic chemicals (A1, A2) or do not release them under realistic conditions (A3). These non-toxic products can serve to direct the design of safer products.

The inter- and transdisciplinary cooperation during the PlastX project has revealed requirements useful to product manufacturers for facilitating their choice-making of a plastic packaging that performs favorably for both human and environmental health (A5). One key factor is a transparent declaration and open accessibility of the ingredients that are contained in a plastic product, as well as their human exposure estimates (A5; Muncke et al., 2017). In cases where competition affects disclosure, independent institutions could evaluate and certify chemical safety such as practiced in the cradle-to-cradle certification (EPEA, 2019). Moreover, it is essential to incorporate all toxicity aspects associated with plastic use into frameworks, such as LCAs, to facilitate the choice of a safe product (Ernstoff et al., 2019).

Improvements in chemical safety form only one component towards a better plastic economy. Another one is to minimize environmental impacts in general. As elaborated in

chapter 2.3, bio-based and biodegradable materials are not the solution here. They neither warrant CO_2 neutrality nor do they solve the world's waste problem. Instead, a fundamental transformation of the current plastic economy is essential. One important aspect is to design products not only for life-in-service but also for end-of-life, or rather, for avoidance of the latter in favor of a potential endless circulation of materials. This circular economy aims to maintain the value of products in order to minimize waste production, raw material and energy use (EPEA, 2019). The Strategy for Plastics in a Circular Economy support this aim and proposes concrete actions to improve recycling and to promote innovation towards circularity, as well as to curb waste and littering (EC, 2018). Although recycling is one important pillar in the transition to circularity, it currently only covers 30% of generated plastic waste in the EU. Of these again, half is exported and treated outside Europe. The main factors responsible for these low recycling rates are the higher price and lower quality of the recycled plastics compared to the un-recycled product (European Parliament, 2018). Therefore, strategies are needed to achieve the European goal of all plastic packaging being recyclable (or reusable) by 2030 (EC, 2018). One important step to achieve higher recycling rates is to reduce the number of polymers and chemicals and, thus, the material diversity of plastics. This will permit efficient single-variety recycling (Hopewell et al., 2009). Simultaneously, it is pivotal to remove hazardous chemicals in order to avoid downcycling due to a reduced safety of the recycled product (A5; Geueke et al., 2018). This emphasizes that aspects of chemical safety are also central in the transition to a sustainable plastic economy.

To illustrate further ways of redesigning and rethinking, it is exemplary outlined how the good '(clean) clothes' can be provided in a sustainable manner. Smart production, such as cutting a garment without creating waste, helps to minimize waste already during production. Moreover, the type and design of a garment can reduce fiber release during use by 80%. Alternative handling, such as less washing, further decreases fiber release and simultaneously reduces energy consumption (Napper and Thompson, 2016). Intentional obsolescence can be avoided if offering a service instead of a product, meaning clean clothes or washing cycles instead of a washing machine (EC, 2019). This example also sets out the interconnectedness of the technical context and societal dynamics and that both need consideration to make change happen.

Ultimately, a change of mindset is essential such that at all stages, plastics are not evaluated based on price, aesthetics and technical functionality alone, but also on criteria such as circularity, as well as environmental and health compatibility (EPEA, 2019).

2.6.5 Actors and responsibilities – Make change happen

It becomes apparent that the plastic system is composed of and influenced by many actors with different prerequisites, interests and requirements. To arrive at a favorable plastic economy, they all need consideration. At the same time, every actor has to acknowledge its responsibilities to achieve effective solutions by collective action. The work on this thesis and in the PlastX project gave some insights into these aspects. They are touched upon in the following section but in a non-comprehensive manner since the consideration of every actor and action is outside the scope of the present work.

Plastic producers and compounders represent important actors in the plastic system. It appears that they rely on and comply with the minimum legal requirements (without scrutinizing them), follow own rules that they have built over the years, or are not even aware of which legal requirements their product has to meet (A5). Changes come with costs and require modifications in behavior. This represents certainly one reason why the plastic industry lags behind in technical solutions. However, there are plastic producers that have expressed the willingness to perform better but at the same time stated that they lack the knowledge for the practical realization of change. Thus, they require professional guidance. Generally, it is essential to make regulations enforceable and to better control their compliance, especially for cases when intrinsic motivation and voluntary commitments to achieve safe and sustainable materials are missing (Daniel et al., 2019). One proposed mechanism to call waste producers to account is an Extended Producers Responsibility (EPR). Besides others, the EPR makes producers pay for the recycling of their products (Prata et al., 2019).

Wholesale and retail are two sectors that co-determine demand. They choose products that are compatible with their business model and decision-making practices, e.g., regarding the appearance (‘will it be sold?’). Accordingly, not only the ecological product criteria but also the associated socio-economic consequences of ‘better’ alternatives need to be made transparent and be preferable. The complex compilation of scientific data, such as in LCAs, makes data difficult to understand and prevents the choice of the ecologically most sustainable and safest material from the outset. Therefore, actors need assistance in understanding and implementing alternatives. For instance, scientists may propose actual applicable options to retail on how to become more sustainable. In this context, a tailored and comprehensible communication is essential (A5).

Regulatory bodies and decision makers need to initiate and drive change. Several regulations and strategies on product authorization (e.g., REACH; EU, 2006), waste (e.g., European Directive on Packaging and Packaging Waste; EU, 1994; European Waste Framework Directive; EU, 2008) and environmental protection (e.g., Water Framework Directive; EC, 2011) address, but do not explicitly mention, plastic pollution. The efficiency of these legislations to reduce plastic-associated risks is unclear. Legislations specific to plastics, which aim at both emission reduction and material safety, are acutely needed (SAPEA, 2019). In order to avoid fast but small scale solutions but to arrive at far-reaching solutions, regulations should consider the systemic nature of the problem (Backhaus and Wagner, 2020). Accordingly, the proposed restriction of intentionally added microplastics to consumer or professional use products by the European Chemicals Agency (ECHA, 2019) and individual bans by EU Member States[20] will only have a modest scope in reducing plastic litter. Furthermore, the task of decision makers is to determine the point at which a risk becomes unacceptable for society and measures have to be taken. They have to adapt to the new challenges that are associated with systemic risks and to make decisions, even if contradicting opinions and uncertainties exist (OECD, 2003).

Policy makers will more easily achieve change with top-down measures, such as (dis)incentives, bans and campaigns, when this approach is complemented with bottom-up mechanisms such as voluntary agreements of the public. Thus, the general public, with their practices and perceptions, requires consideration when developing technical solutions to support their effectiveness. To make behavioral change happen, a combination of knowledge ('what'), motivation ('why') and practical skills ('how') is required (Nisbet and Gick, 2008). Accordingly, facts and information alone are not enough. Instead, to change consumers' behavior, additional predictors of behavior need addressing which encompass concern, personal and social norms, values, identity, attitudes, perceived behavioral control, emotions, habits and awareness (Pahl and Wyles, 2017). As an example, when concern and awareness regarding the impacts of plastics increase, intrinsic motivation to change may exceed extrinsic drivers such as pricing (Pahl et al., 2017). Initial policy interventions, such as plastic bag charges, may still serve to create wider awareness on plastic litter and may 'spillover' to greater behavioral changes. However, that spillover is more likely to occur when motivation is intrinsic rather than extrinsically induced (Thomas et al., 2016). Change can only happen when an alternative behavior becomes a habit (e.g., recycling as the new normal; Andrady et

[20]For instance, France banned primary microplastics in certain cosmetic personal care products (Kentin and Kaarto, 2018).

al., 2015). Another strategy to alter consumer behavior is to modify the view of the general public on the material by attaching value to it and the resources used in it. On the one hand, low cost and marketing support that little value is given to plastic products. On the other hand, consumers and industries do not demand the plastic itself but something connected to it (e.g., fresh food, heat isolation or a medium of communication), thereby, they rarely think of plastics as a matter of consumption (A5; Andrady et al., 2015). Consequently, plastic products must be transformed from a functional tool to the actual object of desire and, thus, not 'being seen' only when improperly managed (A5). One way to achieve greater value is to produce higher quality products with aesthetically pleasing properties in order to create authenticity and to make the material itself a symbol of lifestyle and identity (Bauman, 2007). However, a greater aesthetical value is most probably accompanied with higher costs which make the product less affordable. This draws the attention to the fact that many of the discussed solution options are only applicable if the general living conditions allow for it. People may have no real choice whether, what and how they want to consume or not consume plastics since circumstances (e.g., poverty, inequality) fundamentally deny it. It goes beyond the scope of the present thesis to elaborate on that topic but it should definitively find more consideration when thinking about solutions and change.

Last but not least, scientists can play an important part in the whole process. They have to re-define their roles when dealing with complex and global anthropogenic issues such as plastics. The systemic nature of these issues involves new tasks and requires the cooperation of several scientific disciplines and non-scientific actors. Beyond problem description and provisions of reliable independent evidence, science should also work out and support the implementation of multi-faceted solutions (Backhaus and Wagner, 2020). One important task is to develop a paradigm to characterize plastic-associated risks which considers ecological and societal spheres alike, as well as uncertainties, and can serve as groundwork for action. Furthermore, researchers must acknowledge their social responsibility and communicate their findings in an understandable and comprehensive manner, including the unknowns and uncertainties. Hereby, they prevent unreasonable fear and create the necessary trust as prerequisite to guide policy makers, industries and consumers. Science can be understood as the catalyst for change.

2.6.6 Why care about plastics?

Plastic pollution represents a prototypical, complex and systemic environmental issue of the Anthropocene. Since it is especially visible and tangible, it has received particular attention. One may argue that acting on (micro)plastics redirects scarce monetary and societal resources from more pressing issues (Backhaus and Wagner, 2020), or that the common momentum of desire for change is not to be passed but used to tackle plastic pollution (Kramm et al., 2018). Upon the lessons learnt here, knowledge can be transferred to other issues of the Anthropocene. In addition, tackling the plastic issue itself already aids co-solving further challenges since it creates the framework needed to raise awareness on environmental and sustainability issues and to generate a momentum for changing the current structures and points of view (Völker et al., 2017).

2.7 Final conclusion and recommendations

In the present work a wide range of consumer products were analyzed for their toxicity and chemical composition. Moreover, the drivers of microplastic toxicity were investigated. Therefore, *in vitro* bioassays, *in vivo* ecotoxicological assessments and non-target chemical analyses were combined. In addition, the integration of the experimental findings in the social-ecological research context of the PlastX project allowed placing them into a wider picture. This work lays the foundation for a further evaluation of whether everyday plastics and their alternatives pose a hazard to human and environmental health.

The present thesis provides the following conclusions:

- Plastics contain a great number and diversity of chemicals, many of which are unknown. This hinders the safety assessment of plastics.
- Most everyday plastics contain chemicals that trigger *in vitro* toxicity. Since chemicals also leach under realistic conditions, it is not warranted that human and wildlife exposure to plastic chemicals is safe.
- Bioplastics and plant-based materials do not necessarily represent a better alternative to conventional plastics, especially in terms of toxicity.
- Each plastic product has an individual chemical composition and toxicity. This implies that no generalization on the toxicological or chemical profile of a product is possible based on the material type, the feedstock or biodegradability of a product, nor its application (e.g., in food contact) or its visual appearance. As a result, no clear advice on the least toxic plastic products can be given.
- Irregular microplastics of less-studied polymer types (PVC, PUR and PLA) negatively affect life-history traits of filter-feeding freshwater crustaceans in a plastic type- and endpoint-dependent manner. Either chemicals or the physical properties can drive microplastic toxicity. This depends on the plastic type. Thus, microplastics cannot be treated as homogenous entity when assessing their hazards.
- The lack of knowledge transfer throughout the whole product life cycle withholds relevant information on plastics and their ingredients. In addition, the incompatibility of available knowledge with common practices prevents practical implementation of better alternatives. These factors hinder a safe and sustainable packaging design.

The following recommendations for action towards safer plastic(-like) products, as well as to assess microplastic hazards, can be derived:

- The product-specific and complex chemical composition of plastics, as well as the many unknown compounds, make a product-by-product safety assessment inevitable. Conclusive assessments can be realized by combining whole migrate toxicity testing of the final product with chemical analysis.
- Microplastic research has to reflect the heterogeneity of microplastics in the environment. For instance, less-studied polymer types, including bio-based and biodegradables materials, as well as the chemical and physical complexity of microplastics, need consideration when aiming at a conclusive ecotoxicological risk assessment.
- The introduction of positive lists for starting substances and additives, also comprising information on their properties and safety, can reduce the material complexity and prevents the use of hazardous compounds. To support this aim, transparency regulations and guidelines need to be expanded.
- Adaptions in the polymerization and general processing of plastics can lower or impede the leaching of chemicals from the polymer backbone.
- Non-toxic products are already available on the market that can serve to direct the design of safer products.
- A culture of collaboration and communication over the whole production chain of plastics, including companies, science and the public, is essential. This helps to identify knowledge gaps and to make knowledge more broadly available, understandable and applicable.

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Annex

A1 Benchmarking the *in vitro* toxicity and chemical composition of plastic consumer products

Benchmarking the *in vitro* toxicity and chemical composition of plastic consumer products

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Declaration of author contributions to the publication:

	LZ	GD	TAT	CV	MW
Concept and design	**60%**	–	–	10%	30%
Conducting tests and experiments	**95%**	5%	–	–	–
Compilation of data sets and figures	**90%**	–	–	–	10%
Analysis and interpretation of data	**80%**	–	–	–	20%
Drafting of manuscript	**75%**	1%	1%	5%	18%

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Benchmarking the in Vitro Toxicity and Chemical Composition of Plastic Consumer Products

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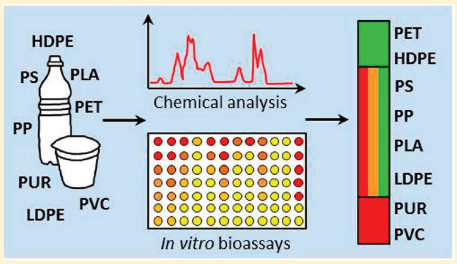
Supporting Information

ABSTRACT: Plastics are known sources of chemical exposure and few, prominent plastic-associated chemicals, such as bisphenol A and phthalates, have been thoroughly studied. However, a comprehensive characterization of the complex chemical mixtures present in plastics is missing. In this study, we benchmark plastic consumer products, covering eight major polymer types, according to their toxicological and chemical signatures using in vitro bioassays and nontarget high-resolution mass spectrometry. Most (74%) of the 34 plastic extracts contained chemicals triggering at least one end point, including baseline toxicity (62%), oxidative stress (41%), cytotoxicity (32%), estrogenicity (12%), and antiandrogenicity (27%). In total, we detected 1411 features, tentatively identified 260, including monomers, additives, and nonintentionally added substances, and prioritized 27 chemicals. Extracts of polyvinyl chloride (PVC) and polyurethane (PUR) induced the highest toxicity, whereas polyethylene terephthalate (PET) and high-density polyethylene (HDPE) caused no or low toxicity. High baseline toxicity was detected in all "bioplastics" made of polylactic acid (PLA). The toxicities of low-density polyethylene (LDPE), polystyrene (PS), and polypropylene (PP) varied. Our study demonstrates that consumer plastics contain compounds that are toxic in vitro but remain largely unidentified. Since the risk of unknown compounds cannot be assessed, this poses a challenge to manufacturers, public health authorities, and researchers alike. However, we also demonstrate that products not inducing toxicity are already on the market.



1. INTRODUCTION

To date, humankind has produced 8300 million metric tons of plastics with an exponentially growing production.[1] From a material perspective, plastics are cheap and versatile materials and, thus, an integral part of our everyday lives. From a chemical perspective, plastic products are complex mixtures of one or more polymers, fillers, and multiple additives, such as plasticizers, flame retardants, stabilizers, antioxidants, and pigments to improve the material's functionality.[2] In addition to these additives, other chemicals are present in plastics, including unreacted monomers, starting substances, and nonintentionally added substances (NIAS, impurities and side or breakdown products).[3]

As most of these chemicals are not covalently bound to the polymer, they can be released at all stages of the plastics' life-cycle via migration to liquids or solids or via volatilization. This can result in a transfer of chemicals in the packed goods (e.g., foodstuff), as well as human (e.g., indoor air and household dust) and natural environments (e.g., water bodies). Accordingly, plastic materials are an important source of human exposure to chemicals.[4] Well-known examples include the plastic monomer bisphenol A (BPA) and phthalate esters used as plasticizers.[5] Their metabolites have been detected in >92 and >98% of the US general population, respectively,[6−8] indicating ubiquitous exposure.

While exposure, hazard, and epidemiological data on few, prominent plastic-associated chemicals, such as BPA, is abundant,[9] it remains challenging to assess the chemical safety of plastics because (1) they comprise a diverse and heterogeneous group of polymers and (2) each product has an individual and complex chemical composition, which (3) often includes unknown compounds. Today, more than 5300 polymer formulations are commercial available[10] and more than 4000 known chemicals are associated with plastic packaging alone.[4] This chemical complexity puts into question current approaches to assess the safety of plastics, especially with regards to food contact materials (FCMs).[11] While the

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risk of starting substances and additives is evaluated prior to the authorization of FCMs in many countries,[12] this approach disregards unexpected and unknown compounds present in the final product (e.g., NIAS), as well as mixture toxicity.[13]

To address these limitations, in vitro and in vivo bioassays can be used to assess the toxicity of the whole migrate leaching from the final product.[14,15] Compared to the chemical analysis of selected target compounds, bioassays integrate the toxicity of mixtures leaching from plastics including known chemicals with unknown toxicity and truly unknown compounds. Further, the chemicals causing toxicity can be identified when coupling bioassays to chemical analysis.[16,17]

Previous studies have demonstrated that plastic FCMs induce in vitro and in vivo toxicity.[14] Since these studies focused on few end points and products, a comprehensive toxicological characterization of plastics is missing. Thus, our study aims at comparing the toxicological and chemical profiles of a range of everyday consumer products made of petroleum-based commodity and bio-based polymers. We hypothesized that the toxicity present in plastics can be benchmarked based on the polymer type. Further, we tested the hypothesis that their chemical signature predicts the toxicity. Finally, we aimed at identifying and prioritizing the chemicals leaching from plastics.

We selected 34 plastic consumer products from the market covering FCMs and non-FCMs made of high-density and low-density polyethylene (HDPE, LDPE), polystyrene (PS), polypropylene (PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyurethane (PUR), and the bio-based polylactic acid (PLA). We extracted these products and analyzed the extracts' baseline toxicity, oxidative stress induction, cytotoxicity, and endocrine activity in vitro. In addition, we performed nontarget, high resolution gas chromatography−mass spectrometry (GC-QTOF-MS) to characterize the chemicals present in plastics and used ToxCast data to prioritize them.

2. MATERIALS AND METHODS

2.1. Sample Selection and Polymer Identification. We selected 34 plastic products (Table 1) covering the polymer types with the highest market share (PP > LDPE > HDPE > PVC > PUR > PET > PS).[18] These petroleum-based materials include plastics with high (e.g., PVC) and low additive content (e.g., PET). In addition, we included PLA as bio-based, biodegradable plastics because these materials are potential replacements for petroleum-based plastics.[19] We selected four or five items per polymer type. Wherever possible, we included packaging products as this sector has the highest plastic demand.[18] We selected high consumption product classes based on their share in municipal waste (containers > plastic wraps > bags and sacks > soft drink bottles).[20] The samples include 20 products with and 14 without food contact. The ratio of FCMs and non-FCMs is different for the polymer types (PS only FCM, PUR only non-FCM). We purchased the products in local retailer stores and confirmed their polymer types (most contained a recycling code) using Fourier-transform infrared spectroscopy (FTIR, PerkinElmer, Spectrum Two, Waltham, Massachusetts). The spectra of the samples can be accessed under DOI: 10.5281/zenodo.3263830. They were compared to reference spectra from our own library and the literature using the software SpectraGryph.[21]

Table 1. Plastic Products Analyzed in This Study

sample	plastic product	FCM[a]
HDPE 1	refillable drinking bottle	yes
HDPE 2	yogurt drink bottle	yes
HDPE 3	bin liner	no
HDPE 4	shower gel bottle	no
LDPE 1	lemon juice bottle	yes
LDPE 2	plastic wrap	yes
LDPE 3	freezer bag	yes
LDPE 4	hair conditioner bottle	no
PS 1	yogurt cup	yes
PS 2	fruit tray	yes
PS 3	vegetable tray	yes
PS 4	plastic cup	yes
PP 1	refillable drinking bottle	yes
PP 2	yogurt cup	yes
PP 3	gummy candy packaging	yes
PP 4	handkerchief packaging	no
PP 5	shampoo bottle	no
PET 1	soft drink bottle	yes
PET 2	yogurt cup	yes
PET 3	oven bag	yes
PET 4	vegetable tray	yes
PET 5	shampoo bottle	no
PVC 1	plastic wrap	yes
PVC 2	place mat	no
PVC 3	pond liner	no
PVC 4	floor covering	no
PUR 1	scouring pad	no
PUR 2	kids bath sponge	no
PUR 3	acoustic foam	no
PUR 4	shower slippers	no
PLA 1	yogurt cup	yes
PLA 2	vegetable tray	yes
PLA 3	shampoo bottle	no
PLA 4	coffee cup lid	yes

[a]FCM: Food contact material.

2.2. Plastic Extraction. Whenever feasible, we used glass or polytetrafluoroethylene consumables to avoid sample contamination and rinsed all materials twice with acetone (pico-grade, LGC Standards) and annealed them at 200 °C for ≥3 h. The content was removed from packaging samples, and the products were rinsed thoroughly with ultrapure water until residues were completely removed. All samples were cut into 0.5−0.8 × 2 cm pieces and foamy products additionally to a thickness of 0.5 cm. Three grams of each were placed in one or two amber glass vials, depending on their volume. After the addition of 20 mL of methanol (99.9% LC-grade, Sigma-Aldrich), samples were extracted by sonication in an ultrasound bath for 1 h at room temperature. We selected methanol because this was the only solvent that did not dissolve any of the polymers. The methanol was transferred into clean glass vials, and 20 μL of the methanol extracts were retained for chemical analysis. After 200 μL of dimethyl sulfoxide (DMSO, Uvasol, Merck) was added as a keeper, samples were evaporated under a gentle stream of nitrogen to a final volume of 200 μL and stored at −20 °C prior to in vitro analysis. Two procedural blanks (PB 1/2) consisting of amber glass vials not containing any sample but only methanol were treated identically to control for a potential contamination. To contextualize the bioassay results, we use "plastic equivalents"

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in such that "1 mg plastic" implies the toxicity extracted from 1 mg of plastic material. Accordingly, 1 μL sample extract corresponds to 15 mg plastic (exception PS 2: 1 μL = 7.5 mg plastic).

2.3. Bioassays. All bioassays were conducted in 96-well microtiter plates with negative controls, solvent controls (DMSO), PB 1/2, and a solvent blank (SB, 20 mL of pure methanol used for sample extraction evaporated to 200 μL of DMSO). Samples, solvent controls, and blanks were diluted 100-fold (baseline toxicity), 200-fold (oxidative stress response), or 480-fold (endocrine activity) with medium, resulting in a maximum final solvent concentration of 1%, 0.5%, or 0.2% (v/v), respectively. Since DMSO solvent controls did not exhibit any effects compared to negative controls in these concentrations, the results for both controls were pooled. Throughout the experiments, none of the controls and blanks induced toxicity. Thus, there was no contamination during sample extraction and analysis (Figure S1).

2.3.1. Baseline Toxicity. The Microtox assay with the bioluminescent bacterium *Aliivibrio fischeri* was performed according to an international guideline[22] miniaturized to a 96-well plate format.[23] In brief, extracts and controls including the reference compound 3,5-dichlorophenol (Table S1 and Figure S2) were analyzed in serial dilutions (1:2 in saline buffer). For extracts, these eight concentrations correspond to 0.18−22.5 mg plastic, except for PS 2 (0.09−11.25 mg plastic), PVC 1 and PLA 3 (further diluted to 2.7 μg plastic). Fifty microliters of *A. fischeri* suspension was added to 100 μL sample. Luminescence was measured prior to and 30 min after sample addition using a Spark 10M microplate reader (Tecan, Crailsheim, Germany).

In accordance with the ISO guideline,[22] the results were corrected for the luminescence in the blanks (empty wells) and for the change in luminescence in negative controls over 30 min, resulting in a relative luminescence inhibition (%). Dose−response relationship curves were derived for each sample using a four-parameter logistic model with the lower and upper plateau constrained to 0 and 100% luminescence inhibition, respectively. Results, from three to five independent experiments with two technical replicates each, are expressed as effect concentration (EC_{20} ± SEM, mass of plastic inducing a 20% luminescence inhibition) and mean effect size ± SEM (luminescence inhibition induced by 22.5 mg plastic). In case an EC_{20} could not be derived, we used an EC_{20} of 25 mg plastic indicating that the EC_{20} is larger than the highest analyzed concentration.

2.3.2. Oxidative Stress Response. We used the AREc32 assay to investigate the induction of an oxidative stress response in the Nrf2/ARE pathway.[24] The AREc32 cell line was obtained from Signosis, Inc. (catalog number SL-0010-NP, Santa Clara, CA, USA) and checked for the absence of mycoplasma contamination (MycoAlert PLUS Mycoplasma Detection Kit, Lonza, Walkersville, USA). The assay was performed as described previously[25] with minor modifications. In brief, 12 000 cells $well^{-1}$ were seeded in 96-well plates. After 24 h, 100 μL medium $well^{-1}$ was replaced by medium containing eight concentrations of the samples serially diluted 1:2 (0.06−7.5 mg plastic except PS 2, 0.03−3.8 mg plastic) or the reference compound *tert*-butylhydroquinone (Table S1 and Figure S2). After 24 h, cell viability and luciferase activity were determined. The former was performed visually by brightfield microscopy (Zeiss, Axiovert 40C)[26] as this was more sensitive than the resazurin assay. If morphological changes (abundance of spherical or dead cells) were apparent, the respective treatment was considered cytotoxic and excluded from further analysis. The luciferase activity was determined immediately after adding 100 μL of 0.015% w/v beetle luciferin potassium salt (Promega, E1601) using a Spark 10M microplate reader. Each sample was analyzed in three to four independent experiments with duplicates each.

We derived dose−response relationships for the induction ratios (IR) using a four-parameter logistic model (lower plateau constrained to 1) to interpolate the plastic mass producing an IR of 2 over the control (EC_{IR2}). In case an EC_{IR2} could not be derived, we used an EC_{IR2} of 8 mg plastic, indicating that the EC_{IR2} is larger than the highest analyzed concentration. The IR at the highest noncytotoxic concentration is also reported.

2.3.3. Endocrine Activity. We used yeast-based reporter-gene assays to investigate the induction of agonistic activity at the human estrogen receptor α (hERα)[27] and antagonistic activity at the human androgen receptor (hAR).[28] The Yeast Estrogen Screen (YES) and the Yeast Antiandrogen Screen (YAAS) were performed as previously described with minor modifications.[29] In brief, samples were diluted 480-fold in medium resulting in a final sample concentration of 3.75 mg plastic equivalents $well^{-1}$. Samples that induced ≥20% cytotoxicity were excluded and reanalyzed in seven additional 1:2 serial dilutions (lowest concentration in the YES, PLA 3 = 3.7 μg plastic, PS 2 = 29.3 μg, PVC 2/PLA 1 = 58.6 μg, and in the YAAS, PLA 3 = 3.7 μg plastic, PP 2 = 14.6 μg, PP 3/PP 5/PVC 2/PLA 1 = 29.3 μg). 17β-estradiol and flutamide served as reference compounds for the YES and YAAS, respectively (Table S1 and Figure S2). To determine the antagonistic activity in the YAAS, 10 nmol L^{-1} testosterone, inducing ∼75% activity, was added. The initial cell density was adjusted to 25 formazin attenuation units (FAU) for YES and 100 FAU for YAAS. After 20 h incubation, we determined the cell density as absorbance at 595 nm on a Spark 10M instrument. After transferring 30 μL $well^{-1}$ to a new 96-well plate, 50 μL of *lacZ* buffer containing 1.5 mmol L^{-1} 4-methylumbelliferyl β-D-galactopyranoside (MUG, Merck, CAS 6160-78-7) and 1 mmol L^{-1} dithiothreitol (Sigma-Aldrich, CAS 3483-12-3) was added. The fluorescence (excitation = 360 nm, emission = 465 nm) was determined after 40 min incubation at 30 °C using a Spark 10M instrument. We also analyzed all samples for autofluorescence prior to the MUG addition and did not observe any. All noncytotoxic samples were analyzed in three independent experiments with eight replicates, each.

Data was processed as previously described to derive the relative cytotoxicity, as well as relative estrogenic and antiandrogenic activities.[30] The limit of detection (LOD) of each experiment was calculated as three times the standard deviation (SD) of pooled negative and solvent controls. Significant differences were calculated for effects > LOD.

Dose−response relationships for cytotoxicity and relative endocrine activity were calculated using a four-parameter logistic function constrained to bottom level of zero (0% cytotoxicity/activity) and for cytotoxicity also a top level of 100%. The respective plastic equivalents inducing 20% cytotoxicity (effect concentration, EC_{20}) were interpolated from the dose−response curves. For the endocrine activity, the EC_{50} was used. To ensure comparability of independent experiments only those experiments were considered in which the dose−response relationship of the reference compound

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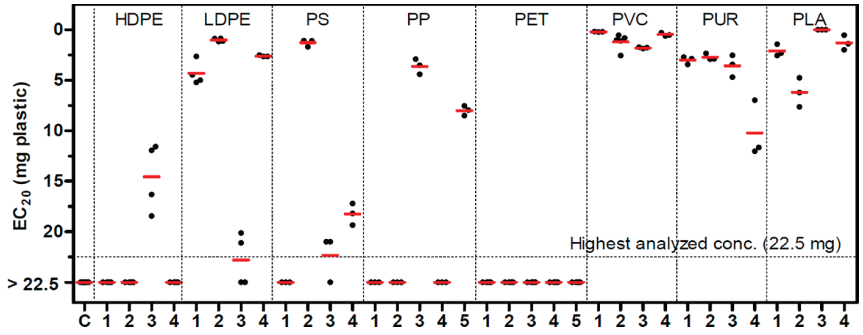

Figure 1. Baseline toxicity of plastic extracts in the Microtox assay. Data is presented as mean EC_{20} for bioluminescence inhibition (lines) from three to five independent experiments (dots) performed with duplicates. The >22.5 indicates that the extracts of 22.5 mg plastic (highest analyzed concentration) did not inhibit the bioluminescence by >20%.

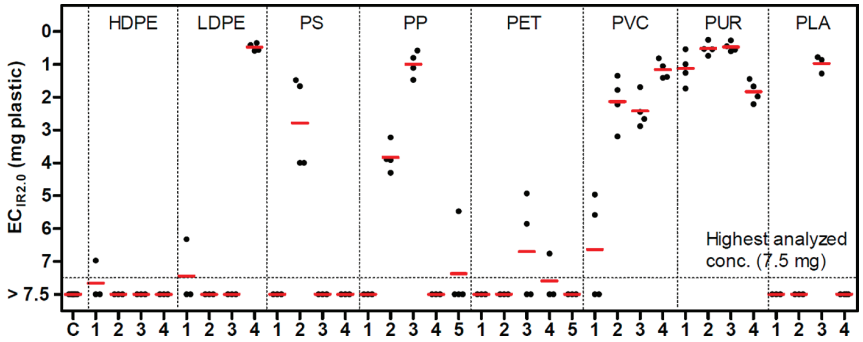

Figure 2. Oxidative stress response induced by plastic extracts in the AREc32 assay. Data is presented as mean EC_{IR2} (lines) from three to four independent experiments (dots) performed with duplicates. The >7.5 indicates that extracts from 7.5 mg plastic (highest analyzed concentration) did not produce an induction ratio of 2 (IR2).

had a $r^2 > 0.9$, a minimal relative luminescence unit >4500, and a maximal >50 000, as well as an EC_{50} next to 6×10^{-11} mol L^{-1} 17β-estradiol (YES) or 2×10^{-5} mol L^{-1} flutamide (YAAS, Table S1).

2.4. Chemical Analysis. Methanolic extracts were analyzed using an Agilent 7890B gas chromatograph with electron ionization and an Agilent 7200 QTOF mass spectrometer (1 μL injection volume, see SI for details). Chromatograms were automatically integrated using Masshunter (selecting peaks with an area ≥ 1% of the largest peak, "features") and compounds identified by comparison of the mass spectra with the NIST 14 library (score ≥ 70) using a nontargeted approach. We refer to the latter chemicals as tentatively identified as we did not use authentic standards to confirm their identity. This corresponds to level 2 of confirmation (probable identification).[31] We removed all tentatively identified compounds found in both PBs from our samples. For each sample and PB, we calculated the sum of all peak areas as indicator for the total abundance of chemicals, the total peak count (features) as indicator for the number of compounds and the relative number of unidentified peaks (score < 70). The raw data from GC-QTOF-MS/MS analysis can be accessed under DOI: 10.5281/zenodo.3263830.

2.5. Data Analysis. We used GraphPad Prism 5 and 7 (GraphPad Software, San Diego, CA) for nonlinear regressions and statistical analyses. To compare two treatments, we used Mann−Whitney tests. A $p < 0.05$ was considered statistically significant.

Out of the tentatively identified chemicals from the GC-QTOF-MS analysis, we selected the five peaks with the largest areas that did not occur in the blanks and queried their CAS numbers in PubChem[32] using R[33] to extract information on the compounds' industrial function according to the Toxic Substances Control Act (TSCA).[34] In addition, we cross-referenced the CAS numbers of all compounds with the database of "Chemicals associated with Plastic Packaging" (CPPdb List A and B)[4] to identify the origin (likely and possibly originating from plastics).

We downloaded the most recent ToxCast database (INVITRODB_V3_SUMMARY from the US EPA)[35] and cross-referenced the CAS numbers of all compounds with oldstyle_ac50_Matrix_180918.csv to filter for tested and active chemicals. We selected the high-throughput assays matching our end points (Table S2) and extracted the respective activity values 50 (AC_{50}, concentration at 50% of maximum activity) for our compounds. Taking a worst-case approach, we calculated the ratios of the lowest available AC_{50} and the largest peak area for each end point. We used the ten compounds with the lowest ratio from each end point to compile a joint list of priority chemicals.

To benchmark toxicity in a heat map, we normalized each effect concentration or level (Tables S3, S4, and S5) to the lowest (0%) and highest value (100%) in the data set. We did the same for data from chemical analysis (Table S6; total peak area, number of all detected peaks, and percent of unidentified peaks).

We performed cluster analyses to compare the toxicological (Microtox EC_{20}, AREc32 EC_{IR2}, and YES/YAAS % relative activity) and chemical signatures of the samples. For the latter, we converted the data from the Agilent instrument to an

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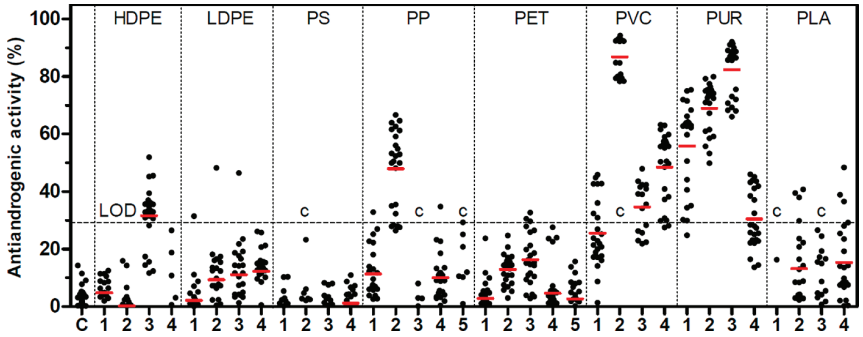

Figure 3. Relative antiandrogenic activity given as relative human androgen receptor inhibition of extracts from 3.75 mg plastic or, if cytotoxic (c), for the highest noncytotoxic concentration (Table S5). Data (n = 24, dots) is presented with means (lines). Mean effects > LOD were considered significant.

mzML format using MSConvertGUI[36] and processed the data using MZmine 2.33[37] to generate a joint peak list containing the peak areas of all masses detected in the samples. We calculated the Euclidean distance between samples and clustered them hierarchically using the "complete linkage" method with the "dist" and "hclust" functions in R.[33]

3. RESULTS

3.1. Baseline Toxicity. The inhibition of bioluminescence in *A. fischeri* is more sensitive than other end points for nonspecific toxicity, such as cytotoxicity in mammalian cells.[38] We observed baseline toxicity for two-third of the 34 plastic extracts (Figure 1). All PVC, PUR, and PLA, as well as three out of four LDPE products inhibited bioluminescence with a high efficiency (low EC_{20}) and effect level (Table S3 and Figure S3). In contrast, none of the PET extracts induced an effect. The baseline toxicity of HDPE, PS, and PP extracts varied with the product.

3.2. Oxidative Stress Response. The AREc32 assay is used to investigate the induction of the Nrf2-ARE regulated oxidative stress response in a human cell line.[24] Fourteen plastic extracts activated this pathway (Figure 2), including all PVC and PUR samples. While PUR extracts (EC_{IR2} = 0.47−1.82 mg plastic) were more efficient than PVC extracts (EC_{IR2} = 1.16−5.27 mg plastic), the effect level was higher for PVC (IR = 2.58−13.6) than for PUR samples (IR = 2.75−3.88, Table S4 and Figure S4). In addition, one LDPE, PLA, PET, and PS sample each, as well as two PP samples, induced an oxidative stress response. Here, LDPE 4 induced the highest effect (IR = 37.0) with a high potency (EC_{IR2} = 0.48 mg plastic).

3.3. Endocrine Activity. To investigate whether plastics contain estrogen receptor agonists or androgen receptor antagonists, we analyzed the samples in reporter gene assays. Four extracts (HDPE 3, PS 1, PVC 2, and PVC 4) activated the estrogen receptor above the LOD (2.33% relative estrogenic activity). However, the estrogenic activity was low for all samples (Table S5 and Figures S5 and S6), except for a place mat (PVC 2). This sample induced the strongest estrogenic activity with up to 27% (at 0.94 mg plastic, Table S5 and Figure S6).

Compared to that, the extracts' antiandrogenic activity (LOD = 29.18%) was more pronounced, with 9 out of the 34 samples inhibiting the androgen receptor by 30−87% (Figures 3 and S7 and Table S5). Here, all PUR extracts, three PVC extracts, and one extract from PP and HDPE were antiandrogenic. As for estrogenic activity, the place mat (PVC 2) induced the strongest effect (EC_{50} = 0.97 mg plastic, 87% receptor inhibition, Table S5 and Figure S7).

3.4. Cytotoxicity. In total, nine extracts were cytotoxic to the cells used in the AREc32 assay (Table S4). Here, PS 2 and PUR 1−3 were most potent with a highest noncytotoxic concentration of ≤1.88 mg plastic. In yeast cells, four extracts (PS 2, PVC 2, PLA 1, and 3) were cytotoxic (Table S5, EC_{20}= 0.05−3.59 mg plastic). In addition, PP 3 and 5 were cytotoxic in the YAAS but not in the YES. The extract of a PLA shampoo bottle (PLA 3) was most potent.

3.5. Comparison of Food and Non-food Contact Materials. To investigate whether FCMs contain a lower toxicity than non-FCMs, we pooled the data from the 20 products with and the 14 products without food contact. We did not observe a significant difference for baseline toxicity and estrogenicity (Figure S8). In contrast, non-FCMs induced a significantly higher oxidative stress response and antiandrogenicity. However, this was not generally true as some individual FCMs were more toxic than non-FCMs made of the same plastic type (e.g., in case of PP, PET, and PVC). Furthermore, we observed a high toxicity for specific food contact articles, including a food wrap (PVC 1, baseline toxicity and antiandrogenicity), a yogurt cup, a food tray, and a coffee cup lid (PLA 1, 2, and 4, baseline toxicity), a gummy candy packaging (PP 3, oxidative stress response), and another yogurt cup (PP 2, antiandrogenicity).

3.6. Nontarget Chemical Screening. To get an overview of the chemical content of the plastic extracts, we ranked them according to the total peak count and area derived from the GC-QTOF-MS data. Overall, we detected between 0 and 194 features per sample. PVC 3 had the largest total peak count and area (Table S6). In total, 15 extracts contained more than 40 peaks, including all PVC, three PUR and three PP products. On the lower end of the spectrum, the PET samples contained a maximum of five features and small total peak areas. Four PVC and two PLA products ranked among the samples with the ten largest total peak areas.

In total, we detected 1411 features. We searched their mass spectra in the NIST database to tentatively identify them. Here, 362 spectra matched a known chemical with a score ≥70 (26% of all compounds, Table S7) corresponding to 260 unique compounds. These represent 18% of all detected chemicals. Out of the 260 unique chemicals, 60 were detected in more than one sample, including 12 compounds that were present in more than three samples (Table S8). Butylated

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Table 2. High Priority Chemicals in Plastics According to Toxicity (ToxCast data) and Abundance in the Samples (Peak Area)[a]

CAS	name	lowest AC_{50} value from ToxCast (μM)				origin	detected in samples
		OX	AA	E	CT		
10482-56-1	α-terpineol	39.5	NA	1.64	0.06	C	LDPE 1/4
112-62-9	9-octadecenoic acid (Z)-, methyl ester	ND	NA	46.3	1.64×10^{-3}	P	LDPE 2, PVC 2
112-63-0	9,12-octadecadienoic acid (Z,Z)-, methyl ester	27.8	53.9	6.93	24.9	p	LDPE 1
112-80-1	oleic acid	100	4.59	33.4	1.00×10^{-5}	P	LDPE 1, PLA 3/4
115-99-1	linalyl formate	60.4	NA	NA	0.17	C	LDPE 1/4
119-61-9	benzophenone	112	NA	5.35	0.24	P	PP 5, PVC 1/3
120-46-7	dibenzoylmethane	4.16	45.3	7.22	52.9	P	PVC 3
128-37-0	butylated hydroxytoluene	49.2	0.11	21	0.08	P	PP 3/5, PVC 2/3, PUR 1/2/4
13466-78-9	3-carene	53.0	NA	92.3	NA	C	HDPE 4, LDPE 1/4
143-07-7	dodecanoic acid	106	14.0	6.85	18.8	P	PLA 3/4
149-57-5	hexanoic acid, 2-ethyl-	NA	52.8	NA	0.83	P	PUR 2
2425-77-6	1-decanol, 2-hexyl-	91.0	NA	14.9	46.1	P	PP 5
26896-20-8	neodecanoic acid	ND	22.21	87.0	ND	P	PVC 3
29761-21-5	isodecyl diphenyl phosphate	18.9	45.2	9.39	1.13×10^{-5}	P	PVC 2/3/4
5392-40-5	citral	68.7	NA	22.2	1.64×10^{-3}	P	LDPE 4
554-12-1	methyl propionate	ND	NA	NA	0.22	C/p	PLA 1/2
55406-53-6	iodopropynyl butylcarbamate	3.20	9.19	24.5	1.86	P	PLA 3
57-10-3	*n*-hexadecanoic acid	NA	NA	37.5	70.4	P	PLA 3/4
57-11-4	octadecanoic acid	NA	12.1	2.30	11.1	P	PLA 4
77-90-7	tributyl acetylcitrate	57.3	38.4	NA	NA	P	PP 3/4, PVC 3/4
7785-70-8	α-pinene	NA	NA	0.73	NA	C	LDPE 4
78-40-0	triethyl phosphate	NA	NA	90.5	1.50×10^{-5}	P	PUR 3
80-54-6	lilial	24.7	NA	25.4	0.02	P	PP 5
84-76-4	1,2-benzenedicarboxylic acid, dinonyl ester	ND	3.40	NA	NA	P	PVC 4
84-77-5	didecyl phthalate	ND	17.3	NA	NA	P	PVC 3
85-68-7	benzyl butyl phthalate	45.1	36.8	6.41	1.65×10^{-3}	P	PVC 4
99-87-6	*p*-cymene	NA	NA	NA	3.68×10^{-3}	P	LDPE 4

[a]Compounds listed in Table S10 were classified as plastic-associated (P). The other compounds were likely associated with plastics (p) or the packed content (C). Note, OX, oxidative stress; AA, antiandrogenicity; E, estrogenicity; CT, cytotoxicity; NA, not active; ND, not determined; one compound (76-25-5) was removed as implausible.

hydroxytoluene (7 detects), 1,7-di-iso-propylnaphthalene (6), methyl isostearate (6), and methyl di-t-butyl hydroxyhydrocinnamate (6) were most common. Interestingly, some chemicals were specific to a certain polymer type with styrene and one benzene present in all PS samples (Table S8 and S9).

3.7. Origin and Functionality of the Detected Chemicals. Regarding their functionality, most of the tentatively identified compounds are classified as food additives and contaminants (13.2%), intermediates (9.9%), solvents (8.6%), process regulators and aids (8.3%), surface-active substances (6.3%), as well as lubricants and lubricant additives (6.3%) according to TSCA (Table S10). Regarding their origin, we cross-referenced our data set with the "Chemicals associated with Plastic Packaging database"[4] and found 57 compounds likely or potentially associated with plastic packaging (see Table S9 for details). These chemicals include monomers (styrene in all PS samples) and additives, such as flame retardants (e.g., triethyl phosphate in sample PUR 3), UV filters (e.g., benzophenone in PP 5, PVC 1/3), and antioxidants (e.g., butylated hydroxytoluene in PP 3/5, PVC 2/3, PUR 1/2/4). Further, we identified the plasticizers decanedioic acid, dibutyl ester (PP 3), tributyl acetylcitrate (PP 3/4, PVC 3/4), bis(2-ethylhexyl) phthalate (DEHP in PVC 2), and didecyl phthalate (DIDP in PVC 3). We also detected seven known NIAS, including 9-octadecenamide (PS 2, PP 4, PVC 2, PUR 2, PLA 3), di-*tert*-butylphenol (HDPE 3, LDPE 2/3), a derivative of benzenepropanoic acid (HDPE 2, LDPE 2/3, PP 3/5, PUR 3), and a di-*tert*-butyl-oxaspirodecadienedione (LDPE 3).

3.8. Toxicity of the Detected Chemicals. We cross-referenced the 260 tentatively identified compounds with in vitro toxicity data from ToxCast. Sixty chemicals (23%) were analyzed in at least one assay for estrogenicity, antiandrogenicity, oxidative stress response, or cytotoxicity (see Tables S11 and S2 for assay information). Thirty-one and 24 chemicals were estrogenic or antiandrogenic in at least one ToxCast assay, respectively. Twenty-five and 52 compounds induced oxidative stress or cytotoxicity, respectively. Regarding the polymers, LDPE (13 chemicals), PVC (11), and PLA (7) contained the most known estrogenic compounds, and PVC (11) and PLA (5), as well as LDPE, PP, and PUR (4), the most antiandrogenic compounds. Chemicals inducing oxidative stress or cytotoxicity were most present in LDPE (11), PVC (9), and PP (6), as well as LDPE (31), PLA (16), and PP (15), respectively.

We compared the lowest AC_{50} values of each compound with its highest peak area in the plastic samples (see Table S11). We use the latter as proxy for the abundance of the chemical in the sample. However, this approach has major limitations because the peak area depends on other parameters than concentration, including volatility and ionizability. We used the ratio of AC_{50} to peak area to prioritize the top ten compounds per end point. In total, 27 compounds had a low ratio of toxicity to abundances (Table 2). On the basis of the

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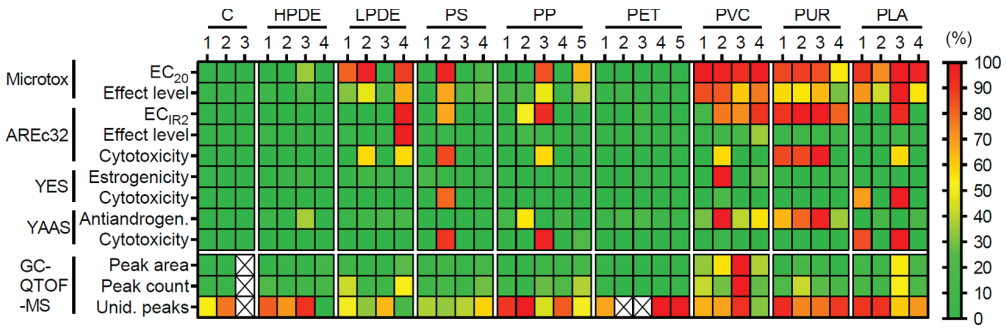

Figure 4. Toxicological and chemical signatures of plastics based on the results of all bioassays and GC-QTOF-MS data (total peak area, number of all detected peaks (peak/feature count), ratio of unidentified peak (unid. peaks)). Controls (C) include procedural blank 1 (1) and 2 (2), as well as the solvent blank (3). Note: EC_{20}, effect concentration inducing 20% baseline toxicity; EC_{IR2}, effect concentration with an induction ratio of 2 over the negative control.

ToxCast data, α-pinene, α-terpineol, and octadecanoic acid were most estrogenic (AC_{50} < 3 μM). Butylated hydroxytoluene and 1,2-benzenedicarboxylic acid, dinonyl ester were the most potent antiandrogens with AC_{50} values < 4 μM. Iodopropynyl butylcarbamate, dibenzoylmethane, and phosphoric acid, isodecyl diphenyl ester induced oxidative stress at the lowest AC_{50} (<20 μM, Table S11). Oleic acid, isodecyl diphenyl phosphate, and triethyl phosphate were most cytotoxic (AC_{50} < 2×10^{-5} μM). Interestingly, 21 out of the 27 compounds affected more than one in vitro end point. Moreover, 21 priority compounds originated from plastics and five were associated with the packed content.

3.9. Comparing the Toxicological and Chemical Signatures of Plastics. A comparison of the toxicological signatures of the products highlights that PVC and PUR affected most end points (Figure 4). PLA was similarly effective, especially regarding the induction of baseline toxicity and cytotoxicity. In contrast, HDPE and PET induced the lowest toxicity across all assays. The signatures of products made from LDPE, PS, and PP are more heterogeneous. Here, some samples were toxic in a range of assays, whereas other products from the same polymer type were not. We performed a cluster analysis to test the hypothesis that the polymer type predicts the toxicity of a material. The samples clustered in three main groups that correspond well to a low, medium, and high toxicity across all assays (Figure S9). All HDPE and PET samples clustered in the low and PUR samples in the high toxicity group. Accordingly, the polymer type may be predictive for the toxicity of these materials. All other polymer types spread across different toxicity clusters indicating that a generalization regarding their toxicity is not possible.

We used the same approach to compare the chemical signatures of the samples and observed no clear patterns regarding the number of detected features and the total peak area (Figure 4). However, this analysis was dominated by PVC 3, which contained by far the most compounds in the highest abundance. The number of unidentified peaks was high across all samples except for most LDPE and PS products. A cluster analysis using the full mass spectral data, including the unidentified peaks, classified the samples according to increasing chemical complexity but did not return distinct clusters (Figure S9). Here, most but not all products made from either PET, HDPE (low complexity), or PS (medium complexity) were chemically very similar. For the other polymer types, chemical signatures clustered widely indicating a low similarity of samples made of the same polymer.

While some products from the low and high toxicity cluster were found to be of low and high chemical complexity, there are some exceptions to this trend. For instance, PUR 1 clustered with the nontoxic samples based on its chemical signature but was highly toxic. Vice versa, the nontoxic HDPE 4 was chemically more similar to the very toxic samples. While there was a general trend for an increased toxicity with higher chemical complexity, chemical, and toxicological signatures do not match. Accordingly, it is not possible to predict the toxicity of a polymer based on chemical analysis.

4. DISCUSSION

4.1. Common Plastic Products Contain Chemicals Inducing in Vitro Toxicity. In previous studies, bioassays have been applied to assess the toxicity leaching from diverse FCMs.[14] However, this is mainly restricted to certain materials and toxicological end points and based on the analysis of packed food or leachates from migration studies. Thus, a comprehensive assessment of the toxicity present in plastic products covering all commodity polymers is absent. In our study, the majority of plastics contained chemicals inducing unspecific toxicity, including baseline toxicity, oxidative stress, and cytotoxicity. Twenty-one out of 34 samples induced baseline toxicity, which in case of the most potent samples translated to cytotoxicity in the other bioassays. Little information is available on unspecific toxicity leaching from plastics. Szczepańska et al.[39,40] reported a strong baseline toxicity migrating from two PE FCMs, as well as baby toys (diverse polymers). In line with our findings, PET-bottled water did not induce baseline toxicity in the Microtox assay[41] or cytotoxicity in MCF7 and PALM cells,[42] as well as HePG2 cells.[43] This implies that PET does not contain chemicals inducing unspecific toxicity. The results on the cytotoxicity of water stored in PET and PVC bottles in murine fibroblasts (L-929) are conflicting.[44] So far, there is no data on plastics containing chemicals triggering an oxidative stress response. While previous reports are sporadic, our results imply that chemicals inducing unspecific toxicity are prevalent in plastic products, especially in those made from PVC, PUR, and PLA.

Our results also show that plastics contain endocrine disrupting chemicals. Here, antiandrogenicity (9 products) was more frequent and potent than estrogenicity (4). Compared to unspecific toxicity, more data is available on the endocrine activity of plastics, mainly on bottled water packed in PET.[14] Estrogenicity has been detected in plastics used as food packaging, medical supplies and labware,[45−48]

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casings of consumer electronics,[17] baby teething toys,[49] and pet toys.[50] Antiandrogenicity was reported in FCMs[48] and baby products.[49,40] Studies with reporter-gene assays compared the endocrine activity of multiple plastic FCMs and confirm our findings that PET does neither contain estrogenic nor antiandrogenic compounds.[47,48] Similar to our study, estrogenicity was less common in PE, PP, and PS[47] than antiandrogenicity.[48] In contrast, Yang et al.[46] reported a widespread estrogenicity leaching from multiple plastic products. Here, 72% of the 455 samples induced a proliferative response in the E-Screen, including products made of PLA, PET, HDPE, PP, and PS. Since our extraction conditions are much harsher than Yang et al.'s, there are only two alternative explanations for the conflicting observations: Either the YES is prone to false-negatives (e.g., because of its lower sensitivity) or the E-Screen is sensitive to false-positives (e.g., because the proliferative response is not exclusively mediated via hERα).

4.2. Toxicity is Less Prevalent in FCMs but Not Absent. Our results indicate that plastic products not intended for food contact induce a higher oxidative stress response and antiandrogenicity compared to FCMs. This may reflect the stricter regulation of chemicals used in FCMs.[12] However, concerns have been raised over the safety of FCMs,[51,14] especially with regards to the migration of unknown chemicals. Along the same line, our study shows that some plastic FCMs contain compounds inducing oxidative stress, estrogenicity, and antiandrogenicity. Importantly, both, FCMs and non-FCMs induced a similar level of baseline toxicity. This underpins the concerns over the adequacy of the current approach for safety assessment of FCMs and implies that bioassays might be more appropriate to assess their safety. Importantly, plastics not intended for food contact can be relevant sources of chemical exposures, too. Humans may be exposed via ingestion (e.g., mouthing behavior), dermal exposure, and inhalation if the chemicals readily migrate. In addition, these chemicals may also affect wildlife, especially in habitats that accumulate plastic litter.

4.3. Plastics Contain a Complex Mixture of Low Molecular Weight Chemicals. Using a nontargeted screening with GC-QTOF-MS, we detected 1411 features in total. While the chemical composition varied with the polymer and the individual product, we detected >40 compounds in 15 samples. This shows that plastic products contain a large number and wide variety of low molecular weight chemicals. So far, the few studies that have used nontarget approaches mainly focus on individual polymers or products. As an example, Dorival-García et al.[52] used GC-Orbitrap-MS and detected 32 and identified 20 compounds in PE-based single-use bags for cell-cultivation. Vera et al.[53] analyzed 26 FCM films made from PP and tentatively identified 74 chemicals. However, as in case with other studies, the total number of detected peaks was not reported. This makes it difficult to evaluate the extent to which the chemical composition of plastics is (un)known. Here, we tentatively identified 260 chemicals out of 1411 features. This demonstrates that most of the chemicals present in plastics (82%) cannot be identified using the NIST database and, thus, remain unknown. Since the health risks of unknown compounds cannot be assessed, this poses a challenge for plastic manufacturers, public health authorities, and researchers alike.

4.4. Toxicological Prioritization of Chemicals in Plastics Is Possible but Remains Fragmentary. Focusing on the tentatively identified compounds, we show that at least 57 chemicals originate from the plastic products in which they are used as monomers, intermediates, solvents, process regulators, and additives. We also detected seven known NIAS. However, the identification of the compounds' origin and function was challenging and hampered by the lack of publicly available data. Accordingly, there is a need to create better chemical inventories for plastics, including NIAS, which will also facilitate the characterization of human exposures to plastic-associated chemicals.

We used ToxCast data to prioritize the detected compounds according to their in vitro toxicity and retrieved high-throughput data for 23% of the chemicals. This highlights that toxicological data is unavailable for most of the known chemicals. Accordingly, we speculate that these 60 compounds are unlikely to explain the toxicity we observed in the plastic extracts. A prioritization resulted in 21 plastic-associated chemicals with high in vitro toxicity, based on ToxCast data, and high abundance in our samples. These include well-known additives (e.g., benzophenone, butylated hydroxytoluene, triethyl phosphate), as well as several compounds that have not received scientific attention but might be toxicologically relevant. For instance, the isomers of decanoic acid that we detected in a range of plastics are estrogenic and antiandrogenic according to ToxCast. Accordingly, this prioritization exercise can help generating hypotheses for future toxicological and epidemiological research.

4.5. Some Polymers Contain More Toxic Chemicals than Others. On the basis of our data, PVC and PUR products contained chemicals inducing the highest toxicity at most end points. In contrast, products made from PET and HDPE induced, if at all, the lowest in vitro effects. As this was true for all samples from those polymer types, we conclude that PVC and PUR generally contain more toxic chemicals than other polymers. This is supported by previous studies with aquatic invertebrates. Here, migrates from PVC and PUR induced the highest acute toxicity compared to other commodity plastics in the freshwater cladoceran *Daphnia magna*,[54] the marine copepod *Nitocra spinipes*,[55] and the barnacle *Amphibalanus amphitrite*.[56] PVC and PUR are known to require large numbers and quantities of additives and have been ranked most hazardous based on their chemical composition.[57] Notably, all PLA products induced strong baseline toxicity similar to PVC and PUR. This demonstrates that this bio-based and biodegradable material, despite being marketed as better alternative, is not necessarily safer than conventional plastics (see ref 58 for review).

For the other commodity plastics, LDPE, PS, and PP, a generalization based on toxicological and chemical signatures is not possible because certain products triggered a range of toxicological end points, whereas others did not. This implies that the toxicity of these products depends on their individual chemical composition, which remains unknown to the public. On a positive note, this also implies that alternative polymer formulations are available on the market not containing the chemicals that induced the toxicity investigated in this study.

4.6. Limitations and Future Directions. Given the diversity of plastics, our analysis of four to five products per polymer type is certainly not representative. Nonetheless and to the best of our knowledge, it represents the most comprehensive study of the toxicity and chemicals present in plastics available, so far. The same is true regarding the in vitro end points we investigated. We selected assays that are well-established, robust, and in parts, standardized. We focused on

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baseline toxicity, oxidative stress, and cytotoxicity, as well as endocrine activity because these are potentially relevant for human health. However, it is important to highlight that our aim was not to draw conclusions regarding the health impacts of plastics but rather to benchmark materials based on their intrinsic toxicity. Along the same line, we extracted plastics as worst-case scenario instead of migration testing with softer solvents (e.g., water). Thus, we expect to see different toxicological and chemical signatures when using more realistic migration conditions. The chemical screening with GC-QTOF-MS is certainly limited because it is selective to semivolatile and nonpolar organic compounds. Accordingly, nonvolatile and polar compounds will be underrepresented in our data. We decided to use GC-QTOF-MS because comprehensive spectral libraries for compound identification are available. However, the NIST database may be limited in their coverage of plastic-associated chemicals, especially NIAS, and the rate of false identifications might be high. A confirmation of compounds of interest using authentic standards can be used to resolve the latter. The same may be true for the ToxCast data, which in addition might be prone to false-positives and -negatives, as recently discussed for PPARγ and RXRα.[59] The only viable strategy to address the limitations of both databases is to perform effect-directed analysis to identify the compounds causing the toxicity present in plastics. In a larger context, we need to approach the challenges of assessing the risks of plastic materials from a new perspective: Acknowledging their chemical complexity is the first step towards developing new scientific and regulatory approaches to improve their safety.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b02293.

> Methodology on chemical analysis, further in vitro toxicity data (baseline toxicity, oxidative stress, cytotoxicity, estrogenicity, antiandrogenicity) of reference compounds and samples, toxicity of FCMs vs non-FCMs, total peak number and area observed in plastic extracts, compounds tentatively identified by NIST database search, list of all compounds and their functionality, as well of those only present in more than three samples and of those associated with plastics, tentatively identified chemicals that induce antiandrogenicity, estrogenicity, oxidative stress response, or cytotoxicity based on ToxCast data, ToxCast assays used to retrieve AC_{50} toxicity values, and hierarchical clustering of plastic extracts according to toxicity and chemical data (PDF)

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L.Z., C.V., and M.W. conceived the study, L.Z. performed the experiments, G.D. performed the chemical analyses, L.Z. and M.W. analyzed the data and wrote the manuscript, and all authors provided comments on the manuscript.

Notes

The authors declare no competing financial interest.

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A2 Are bioplastics and plant-based materials safer than conventional plastics? *In vitro* toxicity and chemical composition

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Conducting tests and experiments	**60%**	40%	–	–
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Are bioplastics and plant-based materials safer than conventional plastics? *In vitro* toxicity and chemical composition

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ABSTRACT

Plastics contain a complex mixture of known and unknown chemicals; some of which can be toxic. Bioplastics and plant-based materials are marketed as sustainable alternative to conventional plastics. However, little is known with regard to the chemicals they contain and the safety of these compounds. Thus, we extracted 43 everyday bio-based and/or biodegradable products as well as their precursors, covering mostly food contact materials made of nine material types, and characterized these extracts using *in vitro* bioassays and non-target high-resolution mass spectrometry. Two-third (67%) of the samples induced baseline toxicity, 42% oxidative stress, 23% antiandrogenicity and one sample estrogenicity. In total, we detected 41,395 chemical features with 186–20,965 features present in the individual samples. 80% of the extracts contained > 1000 features, most of them unique to one sample. We tentatively identified 343 priority compounds including monomers, oligomers, plastic additives, lubricants and non-intentionally added substances. Extracts from cellulose- and starch-based materials generally triggered a strong *in vitro* toxicity and contained most chemical features. The toxicological and chemical signatures of polyethylene (Bio-PE), polyethylene terephthalate (Bio-PET), polybutylene adipate terephthalate (PBAT), polybutylene succinate (PBS), polylactic acid (PLA), polyhydroxyalkanoates (PHA) and bamboo-based materials varied with the respective product rather than the material. Toxicity was less prevalent and potent in raw materials than in final products. A comparison with conventional plastics indicates that bioplastics and plant-based materials are similarly toxic. This highlights the need to focus more on aspects of chemical safety when designing truly "better" plastic alternatives.

1. Introduction

Bioplastics are promoted as an alternative to conventional petroleum-based non-biodegradable plastics. With a production volume of 2.11 million tons in 2018, their market share is very low (1% of all plastics) but expected to increase in the future (European Bioplastics, 2018). The term "bioplastics" is still ill defined. It includes materials made from renewable feedstocks (bio-based, e.g., Bio-polyethylene, Bio-PE), materials supposed to degrade naturally (biodegradable, e.g., polybutylene succinate, PBS), or both (e.g., polylactic acid, PLA; Lambert and Wagner, 2017). Similar materials on the market, such as starch blends, are also defined as bioplastics by European Bioplastics (2018). It is currently unclear whether those and other plant-based materials that are often blends with synthetic materials (e.g., cellulose and bamboo-based materials) fall under that category. Either way, they are produced to fulfill the same function as plastic materials and appear as such to the consumer.

The term "bioplastics" implies that they have similar favorable characteristics as their petroleum-based counterparts (e.g., cheap, lightweight, flexible) but with the positive connotation of "natural" materials. Along that line, they are marketed as more sustainable and benign than conventional plastics. However, little scientific evidence supporting such notion exists. As an example, some biodegradable plastics do not degrade in industrial or natural settings (Haider et al., 2019). When evaluating and improving the environmental performance of bioplastics and plastic alternatives, the main focus is put either on

Abbreviations: Bio-PE, bio-based polyethylene; Bio-PET, bio-based polyethylene terephthalate; EC, effect concentration; hAR, human androgen receptor; hERα, human estrogen receptor α; IR, induction ratio; LOD, limit of detection; PBAT, polybutylene adipate terephthalate; PBS, polybutylene succinate; PHA, polyhydroxyalkanoate; PLA, polylactic acid; SD, standard deviation; UPLC-QTOF-MS/MS, ultra-performance liquid chromatography quadrupole time-of-flight tandem mass spectrometry; YES, Yeast Estrogen Screen; YAAS, Yeast Antiandrogen Screen

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the production stage (e.g., carbon footprint, renewable feedstocks) or at the end of life (e.g., degradability). Currently, the performance during the use phase, such as the human exposure to chemicals are often disregarded when evaluating the materials' sustainability (Ernstoff et al., 2019; Muncke et al. 2020). Along that line, very little is known in terms of the chemical safety of bioplastics, that is the identity of compounds present in the material and their (mixture) toxicity as well as the human exposure to these compound. These gaps in our knowledge are problematic because human exposure to chemicals from bioplastics and plant-based materials will increase with their increasing application.

Compounds intentionally used in plastics include additives such as plasticizers, antioxidants and stabilizers that improve the material's functionality as well as solvents and catalysts that enable production (Hahladakis et al., 2018). In addition, other intentionally (e.g., unreacted monomers) and non-intentionally added substances (NIAS, side or breakdown products) are present (Muncke, 2009). Although the individual compounds will be specific to the material, conventional as well as bio-based and biodegradable plastics can contain all these chemical categories. Additives are particularly relevant for polymers extracted from natural resources, such as starch and cellulose, or from microorganisms, such as PLA, because of their limited physical properties, such as thermal resistance and barrier properties (Beach et al., 2013; Khan et al., 2017). As most of these compounds are not covalently bound to the polymer, they can be transferred to air, solids (e.g., packed good or soil) or liquids (e.g., beverages) in a process called chemical migration. Thus, plastics are a major source of chemical exposures to humans (Muncke et al., 2020) and potentially also terrestrial and aquatic ecosystems.

In our previous study, we demonstrated that the majority of consumer products made of conventional plastics contains chemicals that are toxic *in vitro* (Zimmermann et al., 2019). Interestingly, this was also true for the small set of bioplastics we analyzed. Accordingly, the aim of this study was to investigate whether a broader set of bioplastics and plant-based materials contain chemicals inducing toxicity. We hypothesized that the *in vitro* toxicity of chemicals in bioplastics and plant-based materials is comparable to that of petroleum-based, non-biodegradable plastics and that the toxicity is more pronounced in the finished products compared to the pre-production pellets. We analyzed 43 samples covering nine materials which we grouped according to their feedstock, biodegradability and processing state. We extracted these samples and analyzed the extracts' baseline toxicity, oxidative stress induction and endocrine activity. In addition, we performed non-target high-resolution mass spectrometry (UPLC-QTOF-MS/MS) to characterize the chemicals present in the products.

2. Materials and methods

2.1. Sample and polymer identification

In total, we selected 43 consumer products and raw materials (pre-production pellets, Table 1). The samples cover 27 bioplastics with the highest market share, including materials that are bio-based and biodegradable (PLA, PHA), petroleum-based and biodegradable (PBS, PBAT) as well as bio-based and not biodegradable (Bio-PE, Bio-PET; European Bioplastics, 2018). In addition, we analyzed 16 plant-based materials (starch, cellulose, bamboo). Thirty-one samples held an inscription to be suitable as food contact materials (FCMs). We acquired raw materials, intermediate and final products from local retailers, online suppliers and at a plastics trade fair. We analyzed the products by Fourier-transform infrared spectroscopy (FTIR, PerkinElmer, Spectrum Two, Waltham, Massachusetts; Fig. S1). The spectra of the samples can be accessed under DOI: 10.5281/zenodo.4004763. Using FTIR, we could not differentiate whether the PE and PET used in the products were made from renewable feedstocks or petroleum. Furthermore, we could not always confirm with certainty the material types indicated by the producer, distributor or vendor due to the absence of openly available spectral libraries covering bioplastics and plant-based materials or due to products being blends or composites. Thus, we named and categorized the products based on the origin (bio-based or petroleum-based) and biodegradability of their most prominent component labeled on the product or specified by the supplier. Many products are blends of more than one material (see Tab. S1; Peelman et al., 2013), and we obtained only limited information on the formulation of the samples from the suppliers, despite repeated requests. While in the European Union, monomers, catalysts and additives are regulated under REACH and positive lists exist, producers are not required to publicly disclose the exact chemical formulation of their products (Groh et al., 2019).

2.2. Sample extraction

To avoid sample contamination, we used glass or polytetrafluoroethylene consumables whenever feasible, rinsed all materials twice with acetone (pico-grade, LGC Standards) and annealed glass items at 200 °C for ≥3 h. Additionally, we conducted the sample preparation and the bioassays under a laminar flow hood. For sample preparation, the content was removed from packaging samples and the products were rinsed thoroughly with ultrapure water until all residues were removed. Samples were cut with scissors into 0.5–0.8 × 2 cm pieces. While we were aiming at achieving similar surface areas for all samples, these varied due to the different thickness of the samples. Therefore, we decided to extract the same masses. Three grams of each were placed in one or two amber glass vials, depending on their volume. After adding 20 mL methanol (99.9% LC-grade, Sigma-Aldrich), samples were extracted by sonication in an ultrasound bath for 1 h at room temperature. We used methanol because we aimed at maximizing the extraction of chemicals without dissolving the material completely and to be able to compare our results with our previous study on conventional plastics (Zimmermann et al., 2019). The methanol was transferred into clean glass vials and 200 µL of the methanol extracts were retained for chemical analysis. After adding 200 µL dimethyl sulfoxide (DMSO, Uvasol, Merck) as a keeper, samples were evaporated under a gentle stream of nitrogen to a final volume of 200 µL and stored at −20 °C prior to *in vitro* analysis. In order to avoid the loss of compounds, extracts were not filtered and, thus, may contain nano- and microplastics. Four procedural blanks (PB 1–4) consisting of amber glass vials not containing any sample but 20 mL methanol were treated identically to control for a potential contamination. To contextualize the bioassay results, we use "plastic equivalents" in such that "1 mg plastic" implies the toxicity extracted from 1 mg of plastic. Accordingly, 1 µL extract corresponds to 15 mg plastic. We here report the masses extracted and applied per well of the respective bioassays.

2.3. Bioassays

All bioassays were conducted in 96-well microtiter plates with negative controls (without solvent), solvent controls (DMSO), procedural blanks (PB) and a solvent blank (SB). Samples, solvent controls and blanks were diluted 100-fold (baseline toxicity), 200-fold (oxidative stress response) or 480-fold (endocrine activity) with medium, resulting in a maximum final solvent concentration of 1%, 0.5% or 0.2% (v/v), respectively. Since DMSO did not exhibit any effects compared to negative controls in these concentrations, the results for negative and solvent controls were pooled. In addition, we analyzed solvent blanks (20 mL methanol used for the extraction evaporated to 200 µL DMSO) and procedural blanks (PB, treated exactly like the samples but not containing any material). Throughout the experiments, none of the blanks induced toxicity (see Tab. S3–S6). Thus, there was no contamination during sample extraction and analysis. Pooled blanks (control, C) are presented in the bioassay results (Fig. 1, Fig. S2, S4–S8 and S10).

Baseline toxicity. The Microtox assay with the bioluminescent

Table 1
Bioplastics and plant-based materials analyzed in this study and total number of chemicals features detected by UPLC-QTOF-MS/MS. FCM: Indication that material is suitable for food contact, Type: Raw material (RM), final product (P).

Plastic category	Sample and plastic type	Plastic product	FCM	Type	Number of detected features
Bio-based, biodegradable	PLA 1	Single-use drinking cup	+	P	3755
	PLA 2	Disposable cutlery	+	P	3479
	PLA 3	Film	+	P	8648
	PLA 4	Food tray	+	P	6465
	PLA 5	Coffee capsule	+	P	6121
	PLA 6	Bag for foodstuff	+	P	17,224
	PLA 7	Single-use bottle	+	P	3002
	PLA 8	Film		P	10,958
	PLA 9	Pellet	+	RM	3667
	PLA 10	Pellet		RM	880
	PHA 1	Pellet		RM	614
Petroleum based, biodegradable	PBS 1	Plastic bar		RM	3864
	PBS 2	Food tray	+	P	10,959
	PBAT 1	Waste bag	+	P	15,843
	PBAT 2	Pellet	+	RM	9161
Plant-based	Starch 1	Disposable cutlery	+	P	1065
	Starch 2	Bag for foodstuff	+	P	18,198
	Starch 3	Film		P	15,770
	Starch 4	Film	+	P	16,857
	Starch 5	Pellet	+	RM	9118
	Starch 6	Pellet	+	RM	8325
	Starch 7	Waste bag	—	P	20,965
	Starch 8	Film		P	11,901
	Cellulose 1	Tea bag wrapping	+	P	14,456
	Cellulose 2	Chocolate wrapping	+	P	3378
	Cellulose 3	Cigarette filter	—	P	15,719
	Cellulose 4	Pellet	+	RM	2953
	Cellulose 5	Bag for foodstuff	+	P	20,416
	Cellulose 6	Bag for foodstuff	+	P	14,031
	Cellulose 7	Bag for foodstuff	+	P	17,495
	Bamboo 1	Reusable coffee cup	+	P	5426
Bio-based, non-biodegradable	Bio-PE 1	Bag for foodstuff	+	P	5272
	Bio-PE 2	Wine closure	+	P	1629
	Bio-PE 3	Bag for foodstuff	+	P	n.a.[a]
	Bio-PE 4	Pellet		RM	819
	Bio-PE 5	Food tray	+	P	290
	Bio-PE 6	Film		P	928
	Bio-PE 7	Wine closure	+	P	947
	Bio-PE 8	Pellet		RM	186
	Bio-PE 9	Bag for foodstuff	+	P	19,028
	Bio-PE 10	Film	+	P	13,381
	Bio-PET 1	Reusable bottle	+	P	390
	Bio-PET 2	Box		P	5625

Note: [a] n.a., not analyzed.

bacterium *Aliivibrio fischeri* was performed according to an ISO guideline (ISO 11348-3, 2017) miniaturized to a 96-well plate format (Escher et al., 2008). In brief, extracts and controls including the reference compound 3,5-dichlorophenol (Tab. S2, Fig. S3) were analyzed in serial dilutions (1:2 in saline buffer). For extracts, these dilutions correspond to 0.18–22.5 mg plastic. Fifty µL of *A. fischeri* suspension was added to 100 µL diluted sample. Luminescence was measured prior to and 30 min after sample addition using a Spark 10 M microplate reader (Tecan, Crailsheim, Germany). In accordance with the ISO guideline (ISO 11348-3, 2017), the results were corrected for the luminescence in the blanks (empty wells) and for the change in luminescence in negative controls over 30 min, resulting in a relative luminescence inhibition (%). Dose-response relationships were derived for each sample using a four-parameter logistic model with the lower and upper plateau constrained to 0 and 100% luminescence inhibition, respectively. Results from two to five independent experiments with two technical replicates each are expressed as effect concentration (EC_{20} ± SEM, mass of plastic well^{-1} inducing a 20% luminescence inhibition) and mean effect size ± SEM (luminescence inhibition induced by 22.5 mg plastic well^{-1}). In case an EC_{20} could not be derived, we used an EC_{20} of 25 mg plastic well^{-1} to visualize the data, indicating that the EC_{20} is larger than the highest analyzed concentration.

Oxidative stress response. We used the AREc32 assay to investigate the induction of an oxidative stress response in the Nrf2/ARE pathway (Wang et al., 2006). The AREc32 cell line was obtained from Signosis Inc. (catalog number: SL-0010-NP, Santa Clara, CA, USA). The assay was performed as described previously by Völker et al. (2017), with minor modifications. In brief, 12,000 cells well^{-1} were seeded in 96-well plates. After 24 h, 100 µL medium well^{-1} was replaced by medium containing serial dilutions (1:2 in medium) of the samples (0.06–7.5 mg plastic well^{-1}) or the reference compound *tert*-butylhydroquinone (t-BHT, Tab. S2, Fig. S3). After 24 h, cell viability and luciferase activity were determined. Cytotoxicity was determined via the metabolic reduction of resazurin according to Palomino et al. (2002) with minor modifications. Resazurin sodium salt was dissolved at 0.01% (w/v) in phosphate buffer saline (PBS) and filtered (0.2 µm). Thirty µL resazurin solution was added to each well, incubated for 5.5 h and photometrically measured at 570 and 600 nm (Spark 10 M, Tecan, Crailsheim, Germany). Based on the absorbance of resazurin and resorufin (reduced from resazurin by living cells), the percentage of living cells was calculated. Extracts were considered cytotoxic if they reduced the cell number by > 10% compared to the control. The luciferase activity was determined immediately after adding 100 µL 0.015% w/v beetle luciferin potassium salt (Promega, E1601) using a Spark 10 M microplate reader. Each sample was analyzed in two to four independent experiments with duplicates each. In order to control for the variability

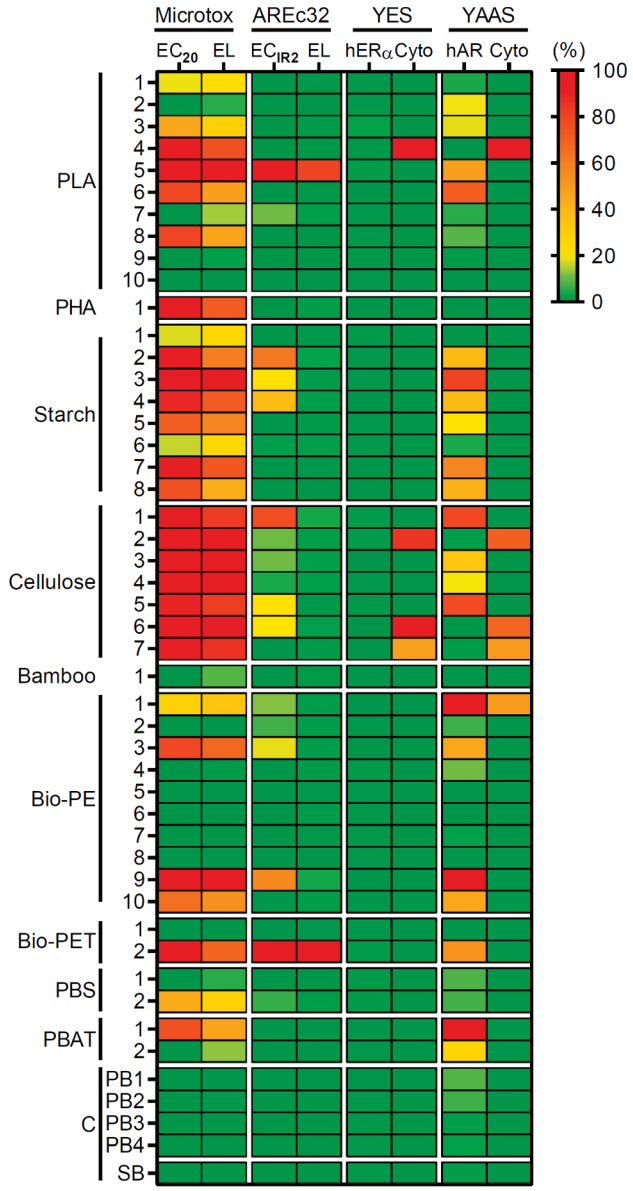


Fig. 1. Toxicological signature of bioplastics and plant-based materials based on baseline toxicity (Microtox), oxidative stress response (AREc32) as well as estrogenic (YES) and antiandrogenic activities (YAAS). The results are presented as effect concentrations (EC_{20}, EC_{IR2}), effect levels (EL), relative receptor activation/inhibition and EC_{20} for cytotoxicity (Cyto). Results are presented as gradient from 0 (green) to 100% (red). The endocrine activities were used as such while the other results were normalized to the lowest and highest effect observed for the respective endpoint. For AREc32 ELs, the highest non-cytotoxic concentrations (Tab. S4) were used. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

between experiments as well as 96-well plates, t-BHT was analyzed on each plate. We only considered those plates on which the t-BHT dose–response relationship was within the 95% confidence interval of the previously verified full-dose response relationship (see Fig. S3). We excluded the concentrations that were cytotoxic in the respective experiment and replicate and derived dose–response relationships for the induction ratios (IR) using a four-parameter logistic model (lower plateau constrained to 1) to interpolate the plastic mass producing an IR of 2 over the control (EC_{IR2}). In case an EC_{IR2} could not be derived, we used an EC_{IR2} of 8 mg plastic well^{-1} to visualize the data, indicating that the EC_{IR2} is larger than the highest analyzed concentration. The IR at the highest non-cytotoxic concentration (across all experiments) is also reported.

Endocrine activity. We used yeast-based reporter-gene assays to investigate the induction of agonistic activity at the human estrogen receptor *α* (hER*α*; Routledge and Sumpter, 1996) and antagonistic activity at the human androgen receptor (hAR; Sohoni, 1998). The Yeast Estrogen Screen (YES) and the Yeast Antiandrogen Screen (YAAS) were performed as previously described with minor modification (Wagner and Oehlmann, 2009). In brief, samples were diluted 480-fold in medium resulting in a final sample concentration of 3.75 mg plastic well^{-1}. Samples that induced ≥ 20% cytotoxicity were excluded and re-analyzed in additional 1:2 serial dilutions (lowest concentration: PLA 4/Cellulose 2: 7.3 µg plastic well^{-1}, Cellulose 6/7: 234 µg well^{-1} and in the YAAS additionally: Bio-PE 1: 469 µg well^{-1}). Additionally, further samples with antiandrogenic effects were also diluted in a 1:2 series (lowest concentration: PLA 5: 7.3 µg plastic well^{-1}, Bio-PE 9/PBAT 1: 234 µg plastic well^{-1} and PLA 6/Starch 3/Cellulose 1/Bio-PET 2: 469 µg plastic well^{-1}). Starch 7 and Cellulose 5 were not analyzed in dilutions since their sample volume was restricted. 17β-estradiol and flutamide served as reference compounds for the YES and YAAS, respectively (Tab. S2, Fig. S3). To determine the antagonistic activity in the YAAS, 10 nmol L^{-1} testosterone, inducing ~75% receptor activation, was added. The initial cell density was adjusted to formazin attenuation units (FAU) of 25 for the YES and 100 for the YAAS. After 20 h incubation, we determined the cell density as absorbance at 595 nm on a Spark 10 M instrument. After transferring 30 µL well^{-1} to a new 96-well plate, 50 µL *lacZ* buffer containing 1.5 mmol L^{-1} 4-methylumbelliferyl *β*-D-galactopyranoside (MUG, Merck, CAS 6160–78-7) and 1 mmol L^{-1} dithiothreitol (Sigma-Aldrich, CAS 3483–12-3) was added. The fluorescence (excitation: 360 nm, emission: 465 nm) was determined after 40 min incubation at 30 °C using a Spark 10 M instrument. We also analyzed all samples for auto-fluorescence prior to the MUG addition and did not observe any. In the YES, all samples were analyzed in two (exception PLA 3: three, Cellulose 2: four) and in the YAAS in two to six independent experiments with eight replicates, each.

Data was processed as previously described to derive the relative cytotoxicity as well as relative estrogenic and antiandrogenic activities (Völker et al., 2016). The limit of detection (LOD) of each experiment was calculated as three times the standard deviation (SD) of pooled negative and solvent controls. Effects > LOD were considered significant. Dose-response relationships for cytotoxicity and relative endocrine activity were calculated using a four-parameter logistic function constrained to bottom level of zero (0% cytotoxicity/activity) and for cytotoxicity also a top of 100%. The respective plastic equivalents inducing 20% cytotoxicity (EC_{20}) were interpolated from the dose–response curves. For the antiandrogenic activity, the EC_{50} was used. To ensure comparability of independent experiments only those experiments were considered in which the dose–response relationship of the reference compound had a $r^2 > 0.9$, a minimal relative luminescence unit < 4500 and a maximal > 50,000 as well as an EC_{50} of 2–30 × 10^{-11} mol L^{-1} 17β-estradiol (YES) or 1–4.8 × 10^{-5} mol L^{-1} flutamide (YAAS, Tab. S2). The mean EC_{50} of 17β-estradiol and flutamide analyzed in each experiment (95% confidence intervals) were 1.26 × 10^{-10} mol L^{-1} (0.23–2.29 × 10^{-10}) and 1.88 × 10^{-5} mol L^{-1} (1.21–2.56 × 10^{-5}), respectively.

2.4. Chemical analysis

Non-target screening of the chemicals extracted from the samples was conducted using ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry/mass spectrometry (UPLC-QTOF-MS/MS) on a Acquity UPLC Waters Liquid Chromatography system (Waters Norge, Oslo, Norway) coupled to a SYNAPT G2-S mass spectrometer (Waters Norge, Oslo, Norway) in positive ionization mode. Two µL methanol extracts (0.15 mg plastic

μL^{-1}) were injected onto an Waters C18 guard column coupled to an Acquity UPLC BEH C18 column (130 Å, 1.7 µm, 2.1 × 150 mm, Waters) with a column temperature of 40 °C. The LC flow rate was 0.2 mL min^{-1} using H_2O with 0.1% formic acid and methanol with 0.1% formic acid as mobile phases A and B, respectively. The gradient started with 80:20% A:B for 0.5 min, then increased to 40:60% at 4.5 min and to 0:100% at 35.5 min. 100% B was maintained until 38.5 min, returned to 20:80% at 39.5 min and equilibrated for 2 min prior to the next injection. The heated electrospray ionization source (positive mode) had a capillary temperature of 120 °C with a spray voltage of 2.5 kV and a sampling cone voltage of 30 V. The desolvation gas flow was 800 L h^{-1}. The mass spectrometer was run in full scan (50–1200 Da) at a resolution of 20,000 with a data-independent MS^E Continuum acquisition with a low collision energy (4 eV) and a high collision energy ramp (15–45 eV). Each sample was analyzed once. LC blanks (methanol) were analyzed approximately after every seventh sample to exclude column contamination. We did not analyze Bio-PE 3 because it contained particulate matter. The mass spectral data of all samples can be accessed under DOI: 10.5281/zenodo.4004763.

2.5. Analysis of chemical data and compound identification

We used Progenesis QI (version 2.3, Nonlinear Dynamics, Newcastle upon Tyne, UK) to analyze the UPLC-QTOF-MS/MS data. In brief, we imported all raw data files (blanks and samples), enabled the search for common adducts (M + H, M + Na, M + H-H_2O, 2 M + Na, 2 M + H, M + H-2H_2O, M + CH3OH + H, M + 2H) and calibrated the *m/z* of all runs using the internal lock mass of leucine enkephalin (556.2766 *m/z*). We automatically aligned the retention times of all runs and performed the peak picking (automatic sensitivity, no predefined peak width).

We exported the resulting feature list to Microsoft Excel for Mac (version 16.35) and compared the maximum raw abundance of each feature in the blanks ($n = 14$) to the raw abundance of the same feature in the individual samples. We filtered for features that were not present in the blanks but in the sample or had a tenfold higher abundance in the sample than in the blank. Based on those results, we identified the ten features that had the highest abundance in each sample as well as the features that were most prevalent across all samples (present in ≥30 samples). In addition, we used the features present in at least one sample per material to compare the different materials and identify those present in more than one material. Using Progenesis QI, we tentatively identified these features by searching all available data sources in ChemSpider with a precursor tolerance of 5 ppm, a fragment tolerance of 10 ppm and a 50% isotope similarity filter. In addition, we performed theoretical fragmentations of the ChemSpider results using the MetaScope algorithm. For each feature, we inspected manually at least the 25 hits with the highest scores and selected the compound identity based on score and plausibility (e.g., by excluding rare elements or salts and focusing on formulas containing C, H, O, N, only). For accepted compounds with a match score > 50, we also performed a PubChem search to retrieve additional information on the use and functionality.

2.6. Statistical analysis of bioassay data

We used GraphPad Prism 5 and 8 (GraphPad Software, San Diego, CA) for nonlinear regressions and statistical analyses. To compare two treatments, we used unpaired t-tests for parametric and Mann-Whitney tests for not normally distributed data. A $p < 0.05$ was considered statistically significant. We performed cluster analyses to compare the toxicological (Microtox EC_{20}, AREc32 EC_{IR2}, and YES/YAAS relative activity) and chemical signatures of the samples. For the latter, we generated a joint peak list containing the abundances of all masses detected in the samples but not in the blanks (see 2.5). We calculated the Euclidean distance between samples and clustered them hierarchically using the "complete linkage" method with the "dist" and "hclust" functions in R (RStudio, 2016).

3. Results

3.1. Baseline toxicity

The bioluminescence inhibition of *Aliivibrio fischeri* is an indicator for baseline toxicity that is more sensitive than other endpoints for unspecific toxicity, such as cytotoxicity in mammalian cells (Neale et al., 2012). Two thirds (67%) of the 43 extracts (Fig. 1, S4 and S5, Tab. S3) induced baseline toxicity. All cellulose-based and starch-based samples as well as the PHA sample inhibited bioluminescence, mostly with a high potency (low EC_{20}) and effect level. The bamboo product did not have any effect in the Microtox assay. The baseline toxicity triggered by the other materials varied with the sample: Six out of ten PLA samples, four out of ten Bio-PE as well as one out of two Bio-PET, PBS and PBAT samples, each, inhibited the bioluminescence.

3.2. Oxidative stress response

In the AREc32 assay, human MCF-7 cells are used to investigate the induction of the Nrf2-ARE regulated oxidative stress response (Wang et al., 2006). Eighteen out of 43 samples (42%) activated this pathway (Fig. 1, S6 and S7, Tab. S4). The Bio-PET 2 extract was most potent (EC_{IR2} = 0.58 mg plastic $well^{-1}$) and had the highest effect level (IR = 64.8), followed by PLA 5 (EC_{IR2} = 1.12 mg plastic $well^{-1}$, IR = 52.5). In addition, six out of seven cellulose-based, four out of eight starch-based, four out of ten Bio-PE, two out of ten PLA and one out of two PBS samples activated the oxidative stress response. However, for most of these samples, effects strongly varied between independent experiments. For example, we measured the strongest variation for Bio-PE 9 with one replicate having an EC_{IR2} of 0.54 and another of > 7.5 mg plastic $well^{-1}$. None of the PHA, PBAT, bamboo-based samples induced an effect.

3.3. Endocrine activity

To investigate whether products contain estrogen receptor agonists or androgen receptor antagonists, we analyzed the samples in yeast-based reporter gene assays. PLA 3 was the only extract that activated the human estrogen receptor α above the LOD (1.56%) with a relative activity of 2.49% at 3.75 mg plastic $well^{-1}$ (Fig. 1, S8, Tab. S5). Four samples (PLA 4, Cellulose 2, 6, 7) were cytotoxic and inactive when analyzed in dilutions (Fig. S9). Compared to the estrogenicity, the extracts' antiandrogenic activity (LOD = 48.6%) was more pronounced, with ten out of 43 samples inhibiting the androgen receptor by 49–98% at the highest non-cytotoxic concentration (Fig. 1, S10, Tab. S5). Here, PBAT 1 (98.0%), Bio-PE 9 (97.4%) and Bio-PE 1 (91.3%) induced the strongest effects. Additionally, two PLA, starch and cellulose samples, each, as well as one Bio-PET extract were antiandrogenic. We also analyzed the dose–response relationships of selected samples that were either antiandrogenic or cytotoxic. Here, PBAT 1 and Bio-PE 9 were most potent with EC_{50} values in the YAAS of 0.40 and 0.39 mg material extracted, respectively (Fig. S11).

3.4. Toxicological signatures of plastics

The toxicological signatures highlight that the chemicals extracted from cellulose and starch samples affected most endpoints, especially baseline toxicity (Fig. 1). In contrast, the bamboo sample and Bio-PE samples contained the lowest toxicity. Nonetheless, four out of ten PE samples had an effect in at least one bioassay, with Bio-PE 9 being very antiandrogenic. The toxicological signatures of PLA extracts were more heterogeneous with PLA 4 and 5 inducing the highest and broadest toxicological response. We observed a similarly heterogeneous picture for the other materials. For example, Bio-PET, PBS and PBAT

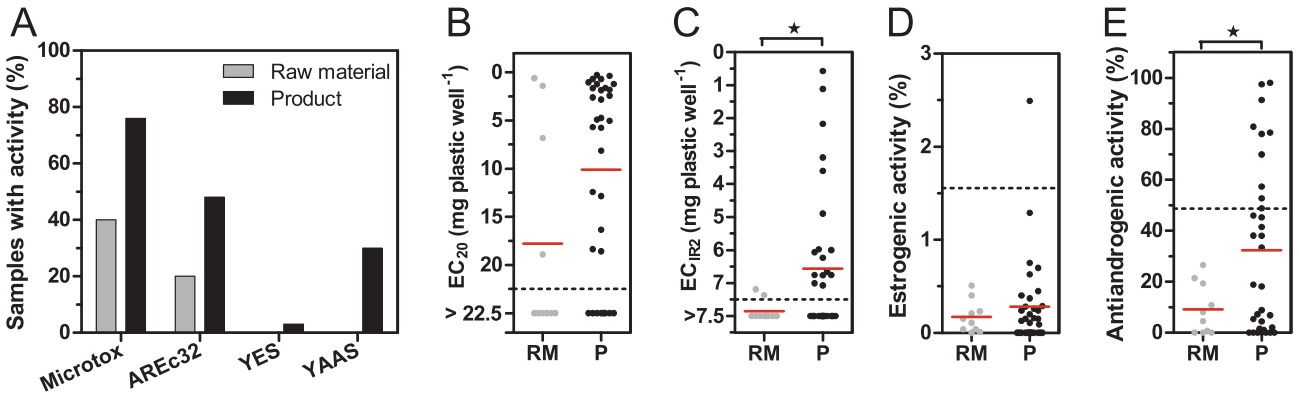


Fig. 2. Toxicity of extracts from raw materials (RM, $n = 10$) compared to final products (P, $n = 33$) with regards to the percentage of active samples (*A*) and the mean effect strengths for baseline toxicity (*B,* Microtox), oxidative stress response (*C,* AREc32), estrogenic (*D,* YES) and antiandrogenic activity (*E,* YAAS). It remains unknown whether the final products were produced from the analyzed raw materials. Each dot represents one sample and red lines the mean. For *D* and *E*, effects are shown for 3.75 mg plastic well^{-1} or, if cytotoxic, for the highest non-cytotoxic concentration (Tab. S5). * $p < 0.05$, unpaired Mann-Whitney test for (*C*) and unpaired *t*-test for (*E*), dotted lines = highest analyzed concentration (*B, C*) or limit of detection (*D, E*). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

comprising one toxic and one non-toxic sample each.

We performed a cluster analysis to test the hypothesis that the material predicts the toxicity of a sample. The samples clustered in four main groups (Fig. S12). The group 2 in the tree includes the samples with the highest toxicity inducing three endpoints. Here, the effect strength was high for at least two and medium for a third endpoint. Groups 1 and 3 cover samples that induced a medium toxicity. The samples in the first group either affected more endpoints or had a higher effect strengths compared to those in the third group. Group 4 comprises the samples with the lowest toxicity affecting no or one endpoint. There was no specific clustering of samples according to their material indicating that the polymer type is not predictive for the toxicity of these materials.

3.5. Comparison of raw materials and final products

To investigate whether final products contain a higher chemical toxicity than the raw materials, we pooled the data from the 33 final products and the ten pre-production pellets. Across all endpoints, more final products induced toxicity compared to the raw materials (Fig. 2). For unspecific endpoints, the percentage of final products having an effect was double that of raw materials, with 78 vs. 40% of the samples inducing baseline toxicity and 48 vs. 20% of samples inducing an oxidative stress response. None of the raw materials contained estrogen-like or antiandrogenic chemicals, whereas 30% of the final products were antiandrogenic. Regarding the mean effect level, final products induced stronger toxicity on all endpoints. However, two raw materials induced a baseline toxicity that was as high as that of the most toxic final products.

3.6. Comparison of bioplastics and plant-based materials with conventional plastics

To analyze whether bio-based and/or biodegradable materials contain chemicals that are less toxic than those in conventional (petroleum-based, non-biodegradable) plastics, we pooled the data from all samples analyzed here and compared it to the data from our previous study in which we tested 30 conventional plastics in exactly the same way as in the present study (Zimmermann et al., 2019). The proportion of samples inducing toxicity was the same for the bio-based/biodegradable materials as for the conventional plastics. A slightly higher percentage of bioplastics and plant-based materials compared to conventional plastics induced baseline toxicity and a slightly higher percentage of conventional plastics had an endocrine activity (Fig. 3). The mean effect strengths of bioplastics and plant-based materials were comparable with conventional plastics across all endpoints, except for estrogenicity which was induced significantly stronger by conventional plastic than bio-based/biodegradable materials. However, this difference was mainly driven by one PVC extract with a relative estrogenic activity of 27.1%.

Comparing petroleum-based plastics with their direct bio-based counterparts, for PE a higher number of bio-based samples induced oxidative stress. However, it was a petroleum-based PE that was most effective (LDPE 4: $EC_{IR2} = 0.48$ mg plastic well^{-1}). More bio-based PE extracts inhibited the androgen receptor and did so with a higher efficiency (up to 97.4%). Interestingly, none of the five conventional PET extracts induced relevant toxicity but one out of the two Bio-PET samples did.

3.7. Chemical features

In total, we detected 51,677 chemical features across the 14 blanks and the 42 samples. Filtering for features that had at least a tenfold higher abundance in samples compared to the blanks, resulted in a total of 41,395 features in all samples. The individual samples contained between 186 and 20,965 features (Tab. 1). Thirty-four samples had > 1000 features each with Starch 7 (20,965 features), Cellulose 5 (20,416) and Bio-PE 9 (19,028) containing the highest numbers. On the other end of the spectrum, Bio-PE 8 (186), Bio-PE 5 (290) and Bio-PET 1 (390) contained the least chemical features.

3.8. Chemical similarity of materials

We compared the similarity of chemical features within and between materials. In total, between 5811 and 31,727 different features were detected per material (of which at least two products were analyzed). When investigating whether features were shared among multiple samples per material, it became clear that most were unique to one sample (Fig. 4A). For instance, about half of all features detected in PLA and Bio-PE were present in only one but not the other samples of the same material. This was less pronounced for starch and cellulose with about 30% of all features being unique to one sample per material. Here, a higher number of features was detected in multiple samples. For instance, 11% of all features were shared by five samples/material. Less than 1.1% of all features detected in a material was present in all samples of that material, corresponding to 285 features for PLA, 110 for starch, 257 for cellulose and 0 for Bio-PE.

Taking a similar approach to compare the features present across

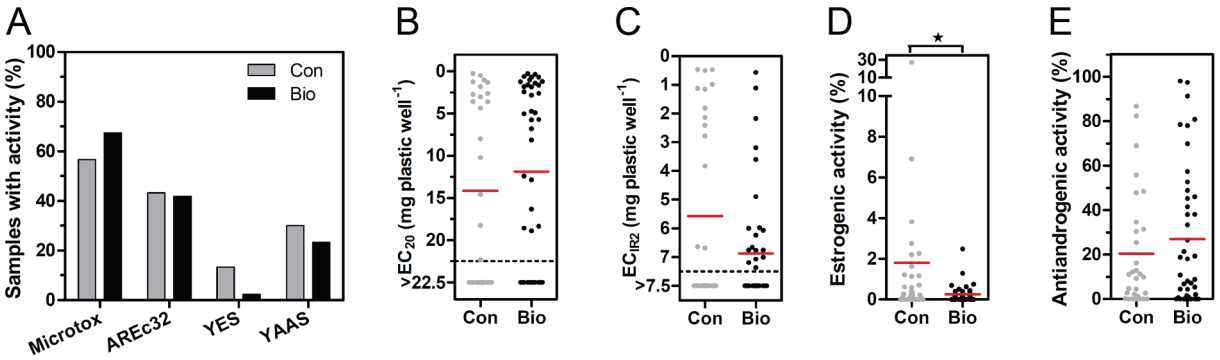


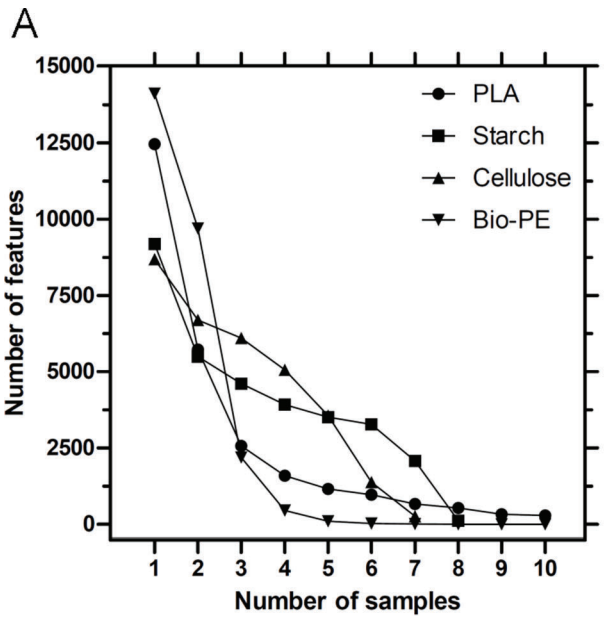
Fig. 3. Toxicity of extracts from conventional, petroleum-based (Con, $n = 30$) compared to bioplastics and plant-based materials (Bio, $n = 43$) with regards to the percentage of active samples (*A*) and the mean effect strengths for baseline toxicity (*B,* Microtox), oxidative stress response (*C,* AREc32), estrogenic (*D,* YES) and antiandrogenic activity (*E,* YAAS). Each dot represents one sample and red lines the mean. For *D* and *E*, effects are shown for 3.75 mg plastic well^{-1} or, if cytotoxic, for the highest non-cytotoxic concentration (Tab. S5). * $p < 0.05$, unpaired Mann-Whitney test, dotted lines = highest analyzed concentration. Toxicity data for conventional materials are taken from Zimmermann et al. (2019). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

different materials, we found that a total of 37 features was present in all materials (i.e., in at least one sample per material). Whereas materials with few features (PHA, bamboo, Bio-PET) shared little chemical similarity with the other materials (Fig. 4B), PLA, starch, cellulose, Bio-PE, PBS and PBAT shared more than one third of all features. PLA and starch, starch and cellulose, starch and Bio-PE as well as cellulose and Bio-PE shared at least two thirds of all detected features (each combination shared > 20,000 features). Further, a cluster analysis using the abundance of all features did not return distinct clusters for the materials (Fig. S13). In some cases, two samples from the same material clustered closely, implying similar chemical signatures. However, in most cases the similarity between materials was higher than within materials.

3.9. Tentatively identified compounds

We tentatively identified the most prevalent features across all samples (i.e., most often detected) and the most abundant features in each sample (i.e., highest intensity). In total, 42 out of the 45 chemical features present in at least 30 samples were identified by the MetaScope algorithm (Tab. S6). The most prevalent feature (*m/z* 641.6915, charge 2+) was detected in 37 samples but remained unidentified. The second most prevalent feature (in 35 samples) is a benzofuran carboxylate with a relatively high match score. However, upon comparison with the PLA oligomers described by Ubeda et al. (2019) this feature appears to be a cyclic lactic acid oligomer ($((C_3H_4O_2)_n$ with $n = 6$). Interestingly, two other compounds also share spectral similarities with PLA oligomers (Tab. S6). Three compounds had a match score > 50: 4-Amino-6-(2-furyl)-2-[2-(4-morpholinyl)-2-oxoethyl]-3(2H)-pyridazinone, (2Z)-4-Methyl-2-pentene-2,3,4-tricarboxylic acid and 2,3,4-Tri-O-acetyl-6-O-(2-methoxy-2-oxoethyl)-alpha-D-galactopyranose (present in 30 samples, each). PubChem did not contain any relevant information on the origin or use of these chemicals.

The ten most abundant features per sample comprised 294 different features indicating some overlap between samples. Out of these, we tentatively identified 271 compounds (Tab. S7). Twenty-six had a score



B

# feat.	PLA 26,258	PHA 614	Starch 32,164	Cell 31,727	Bam 5,426	Bio-PE 26,566	Bio-PET 5,913	PBS 13,049	PBAT 19,188
PLA	-	1.7	65.5	60.5	18.4	59.6	16.9	42.7	53.6
PHA	437	-	1.6	1.6	2.3	1.8	2.3	1.8	1.8
Starch	23,112	510	-	69.2	16.0	65.5	15.6	38.2	54.3
Cell	21,856	508	26,141	-	15.8	62.9	15.7	36.3	50.2
Bam	4,922	134	5,191	5,063	-	17.9	13.3	21.7	20.7
Bio-PE	19,731	476	23,239	22,500	4,865	-	17.7	39.6	59.1
Bio-PET	4,657	145	5,136	5,094	1,330	4,892	-	16.2	18.5
PBS	11,761	245	12,486	11,926	3,290	11,235	2,646	-	44.3
PBAT	15,856	341	18,067	17,013	4,218	16,995	3,926	9,895	-

Fig. 4. Number of chemical features plotted according to the number of samples per material it is detected in (*A*) and number of features shared between materials (*B*). In *B*, features are considered that have been detected in at least one sample per material (sum given as # feat.). The lower left section represents the number of shared features, the upper right section their percentage of all features detected in the combination of materials. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of > 50, including N,N'-1,4-Butanediyldihexadecanamide (used as plasticizer and in coatings/paints), 2-Ethoxyethyl hexadecyl (2E)-2-butenedioate (a fumaric acid that may be used as monomer; ECHA, 2020), 2,2′-(Tridecylimino)diethanol (CAS 18312-57-7, a surface active agent that migrates from PP packaging; Aznar et al., 2012). Some of the remaining compounds may be of natural origin (microbial, fungal or plant) but did not have relevant information regarding their origin/use or their identification was implausible. Other notable compounds with a lower match score are the lubricant and plastic additive N,N'-ethylenebis(palmitamide) (CAS 5518-18-3, detected in PLA; NCBI, 2020), the NIAS 1,6,13,18-tetraoxacyclotetracosane-2,5,14,17-tetrone (CAS 141850-18-2, detected in PLA, starch, Bio-PE and PBAT), the plastic additive erucamide (CAS 112-84-5, detected in starch, cellulose and Bio-PE), the antioxidant Irganox 1076 (CAS 2082-79-3, in Bio-PE), and the antioxidant degradation product tris(2-nonylphenyl) phosphate (CAS 26569-53-9, in Bio-PE). Interestingly, some of the top 10 compounds were not unique to one but also detected in other samples in high abundances including ones made of different materials (Tab. S7). As in case of the most prevalent features, some of the top 10 abundant features in PLA shared similarities with PLA oligomers. Four of those were probably cyclic lactic acid oligomers with $n = 6–9$ based on their mass spectra (Ubeda et al., 2019).

Regarding the features that were present in all material types, we tentatively identified 30 out of the 37 (Tab. S8). The seven features with a match of > 50% were 3-Pyridinylmethyl {4-[5-({4-[2-(4-morpholinyl)ethoxy]benzoyl}amino)-1H-pyrazol-3-yl]benzyl}carbamate, 1-(Bicyclo[2.2.1]hept-2-yl)-3-(3-chloro-4-pyridinyl)acetone, methyl (2E,4E,6S,8E,13R)-13-acetoxy-6-hydroxy-2,4,8-tetradecatrienoate, N-[4-(2-Furyl)-4-hydroxy-2-butanyl]-1-(3-methylbutyl)-1H-1,2,3-triazole-4-carboxamide, S-[(2E,6E)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-yl] methanesulfonothioate, 1-[4-Hydrazino-6-(1H-1,2,4-triazol-1-yl)-1,3,5-triazin-2-yl]-3-pyrrolidinecarboxamide and (2S,3R,4S,5S,6R)-3,4,5-Trihydroxy-6-(hydroxymethyl)-2-[4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl]tetrahydro-2H-pyran-2-carboxamide. PubChem did not contain any relevant information regarding their origin, function or use. Interestingly, the tentatively identified compounds did not include per- and polyfluoroalkyl substances (PFAS).

4. Discussion

4.1. Bioplastics and plant-based materials contain chemicals inducing in vitro toxicity

Bioplastics and plant-based materials are promoted as more sustainable alternative to conventional, petroleum-based non-biodegradable plastics (Lambert and Wagner, 2017). However, currently we do not know whether they also represent a safer alternative with regards to the chemicals they contain, including the hazard and human exposure to these compounds. While knowledge on both is required to arrive at a risk-based assessment, we focus on the toxicity in this study. We extracted the materials as a worst-case scenario to generate first insights into the hazard of the mixture of extractable compounds. Using this approach, we demonstrate that out of the 43 products 29 contained chemicals that induced baseline toxicity, 18 that induced oxidative stress, 10 antiandrogenicity and one estrogenicity. This demonstrates that a range of bio-based and/or biodegradable materials, most of them used as FCMs, contain chemicals that are toxic *in vitro*. While we cannot rule out the presence of nano- and microplastics in the samples, their concentration would have been low due to the dilution in the bioassays. Thus, we believe that the observed toxicities are largely caused by the extracted chemicals.

While a systematic assessment is currently missing, previous research also reported *in vitro* toxicity of bioplastics and other bio-based materials. As an example, cellulose-based materials induced cytotoxicity in mouse fibroblasts (Dang et al., 1996). Up to date, research has mainly focused on the unspecific toxicity of PLA. Accordingly, chemicals leaching from different PLA materials used for medical implants inhibited bacterial bioluminescence (Ramot et al., 2016; Taylor et al., 1994) whereas migrates of PLA-clay nanocomposites used in food packaging were not cytotoxic in human cell lines (Maisanaba et al., 2014). This product-dependent variation of toxicity, even if made of the same material type, corresponds to our findings. For instance, a coffee capsule (PLA 5) but not a single-use bottle (PLA 7) induced *in vitro* toxicity.

Since bioplastics and plant-based materials are often applied in agriculture and horticulture (European Bioplastics, 2018), many studies investigate their *in vivo* toxicity, especially with regards to terrestrial ecosystems. Here, aqueous extracts of pure PLA and PLA-nanoclay induced genotoxicity in the onion *Allium cepa* (Souza et al., 2013). Phytotoxic effects were also observed for leachates of starch-based bags affecting plant germination (Balestri et al., 2019) or whole costal dune vegetations (Menicagli et al., 2019). Studies comparing different materials indicated a material-dependent toxicity of biodegradable materials used in agriculture in plants (Serrano-Ruíz et al., 2018) and soil bacteria (Adhikari et al., 2016). For instance, PLA but not PBS and PBS-starch affected nitrogen circulation activity of soil bacteria (Adhikari et al., 2016). Studies on the toxicity of polyhydroxybutyrate-based materials (PHB) are currently limited to freshwater species. PHB and PBAT leachates reduced the survival of *Daphnia magna* already after 48 h of exposure (Göttermann et al., 2015).

While previous reports are sporadic and predominately focus on PLA, our results imply that chemicals inducing unspecific toxicity are prevalent in all types of bio-based and/or biodegradable products, especially in those made of the natural polymers starch and cellulose. Our results also indicate that these materials contain endocrine disrupting chemicals, with antiandrogenicity being more frequent and potent than estrogenicity. These finding, along with the absence of systematic research, stress that analyzing the chemical toxicity of bioplastics and plant-based materials, especially of materials other than PLA, should be prioritized in future research. This can be achieved by combining bioassays with analytical chemistry (Bergmann et al., 2020; Groh and Muncke, 2017; Veyrand et al., 2017) and embedded in a green chemistry approach that aims in avoiding the use and generation of hazardous substances. As an example, Bandyopadhyay-Ghosh et al. (2018) synthesized a novel polysaccharide biopolymer that did not induce baseline toxicity or genotoxicity.

4.2. Bioplastics and plant-based materials contain a complex mixture of chemicals

Using a non-target screening with UPLC-QTOF-MS, we detected 41,395 chemical features across 42 samples and 186–20,965 features in the individual samples. While products made of starch and cellulose contained the highest number of features (typically > 10,000), the number of substances generally varied from product to product. Most samples (80%) contained more than 1000 chemical features, illustrating the large number and variety of low molecular weight chemicals present in bio-based and/or biodegradable products.

Only few other studies apply non-target analysis to examine compounds in conventional plastics or their “biological” counterparts and the number of detected features is hardly ever reported. As an exception, Aznar et al. (2019) detected 37 non-volatile chemicals in pellets and films made from a PLA/Bio-PE blend using UPLC-QTOF-MS. Bradley (2010) performed a more comprehensive migration study of 13 starch, cellulose, PLA, cassava and bagasse samples and detected up to 32 and 29 compounds using GC-MS and LC-TOF-MS, respectively. Using other instruments and settings, those studies cannot directly be compared to ours. Thus, we probably detected more compounds because we used a different data analysis strategy and considered all features present in the samples with an at least 10-fold higher abundance than in the blanks. Furthermore, the choice of extraction technique (e.g., type of solvent, temperature and duration) will affect the

composition of detected chemicals.

In any case, our results clearly show that bioplastics and similar plastic alternatives contain a strikingly high number and variety of chemicals. While not all of these are relevant for human exposure or the environment, this highlights the challenges we face when aiming to assess the chemical composition and safety of plastics and other synthetic materials, especially when dealing with FCMs (Muncke et al., 2020).

4.3. Chemicals present in bioplastics and plant-based materials

As the non-target analysis resulted in a large number of chemical features, we focused on identifying the compounds that were most prevalent across samples and materials as well as the ten compounds with the highest abundance in individual samples. The *in silico* fragmentation of all corresponding candidates from PubChem resulted in the tentative identification of circa 94% of these chemical features. While this appears promising, care should be taken when interpreting the results. As an example, some of the most prevalent and abundant features in PLA were probably oligomers of lactic acid (Ubeda et al., 2019) and not the compounds identified by the MetaScope algorithm. Likewise, some of the features were identified as pharmaceuticals or natural products which we do not expect to occur in our samples. This highlights the challenges of unknown analysis: General chemical databases often do not cover chemicals used in the manufacture of (semi) synthetic polymers making a query of empirical or theoretical spectra difficult.

These limitations notwithstanding, we tentatively identified a range of plausible compounds in bioplastics and plant-based materials. We found a number of plastic additives, including butanediyldihexadecanamide, ethylenebis(palmitamide), erucamide and Irganox 1076 as well as NIAS, including tetraoxacyclotetracosane-tetrone, a migrate from PE packaging (Sage et al., 2018) that is very similar to a NIAS found in biodegradable packaging (Canellas et al., 2015) and tris(2-nonylphenyl) phosphate (in Bio-PE) which is a degradation product of the antioxidant tris(nonylphenyl) phosphite (TNPP) and has been detected in PE (Celiz et al., 2020).

While this creates some confidence in our identification approach, the need to improve databases and workflows cannot be overstated. This is important because (1) we know even less about the chemical composition of starch-, cellulose- and other plant-based materials than bioplastics and (2) manually curating the identification of thousands of features is not feasible. To overcome these challenges, we need to develop community-sourced spectral databases (as in case of environmental pollutants, e.g., NORMAN network) and suspect lists (as for plastic food packaging; Groh et al., 2019).

4.4. Some materials contain more toxic chemicals than others

Based on our results, the chemicals present in the products made of the natural polymers starch and cellulose were toxic on most endpoints. All starch and cellulose products induced baseline toxicity and many contained antiandrogenic compounds. This indicates that the chemicals used in these materials trigger a stronger *in vitro* toxicity than others. Nevertheless, some extracts of PLA and Bio-PE as well as of the materials of which we analyzed only few samples (PHA, Bamboo, Bio-PET, PBS and PBAT) also induced a range of toxicological endpoints, whereas others did not. Here, a generalization, in such that individual materials would induce a specific toxicological signature, is not possible. Instead, the toxicity of these products rather depends on their individual chemical composition. This is supported by our cluster analysis and mirrors our findings on conventional plastics (Zimmermann et al., 2019). Accordingly, we are facing a similar heterogeneity in terms of toxicity in conventional plastics and bio-based/biodegradable materials alike.

On a more positive note, six out of ten Bio-PE products did not contain toxic chemicals. This implies that bio-based PE formulations are available on the market not containing the substances that induced *in vitro* toxicity. Again, this corresponds to our previous findings on products made of conventional PE (Zimmermann et al., 2019). Here, half of the products were nontoxic in the same bioassays. This is plausible because changing the carbon source of the monomers will only minimally change the chemical composition of the polymer. While some impurities may be different, the reaction by-products and additives will remain the same. Accordingly, shifting from petroleum- to plant-based monomers will probably not affect the toxicity present in the finished material. Such considerations may, however, not apply to Bio-PET. While we did not detect any relevant toxicity in conventional PET (Zimmermann et al., 2019), one of the two Bio-PET samples induced baseline toxicity, triggered an oxidative stress response and was antiandrogenic. Whether this is caused by chemicals specifically used in bio-based PET formulations remains to be investigated.

4.5. Raw materials are less toxic than final products

Across all analyzed endpoints, toxic chemicals were less prevalent and potent in raw materials than in final products. Due to a lack of product information, we do not know whether the analyzed raw materials correspond to the final products. Still, our results indicate that during the conversion of the raw material to the finished product (compounding) new substances are added or generated. This hypothesis is supported by the number of chemical features we observed. Here, we detected overall fewer chemical features in raw materials than in final products of the same material (Tab. 1). As an example, Bio-PE pellets 4 and 8 contained 819 and 186 chemical features, respectively, whereas all but one analyzed Bio-PE product contained more than 900 features. In contrast, the extrusion of bioplastic pellets to a film did not generate new compounds (Aznar et al., 2019). Here, studies analyzing the toxicity of the same raw material and the corresponding finished products can help clarify this question.

4.6. Bioplastics and plant-based materials are not safer than conventional plastics

In our previous work, we analyzed mainly petroleum-based plastics and found toxicity in 67% of the conventional plastics (Zimmermann et al., 2019). Since their bio-based and/or biodegradable counterparts are promoted as sustainable alternatives, we were interested in whether they are indeed safer from a chemical perspective, that is whether they contain less toxic chemicals. Just as for conventional plastics, we detected *in vitro* toxicity in 67% of the bio-based/biodegradable samples using the same bioassays. There were even more bioplastics and plant-based materials than conventional products that triggered baseline toxicity. Regarding effect levels, we detected no significant differences for all toxicological endpoints except for estrogenicity which was less pronounced in bio-based/biodegradable products. To the best of our knowledge, there is no other study that compares the *in vitro* toxicity of conventional plastics and the bio-based/biodegradable alternatives. However, reports on the phytotoxicity indicate that both, starch-based and HDPE bags, released compounds that impaired seedling growth and plant interactions (Balestri et al., 2019; Menicagli et al., 2019). Thus, in this scenario, the chemicals present in natural and synthetic polymers induced a comparable chemical toxicity.

Importantly, the performance and sustainability of bioplastics and plant-based materials cannot be evaluated based on toxicity alone. Here, other environmental (e.g., land and pesticide use, greenhouse gas emissions) and societal impacts (e.g., competition with food production) also need to be taken into account. As life cycle assessments and similar frameworks tend to focus on the latter aspects, an evaluation of the environmental performance and safety of new materials needs to expand to the release of chemicals and particles (e.g., nanoplastics) as well (Ernstoff et al., 2019; Muncke et al., 2020). Only when taking such

holistic view can we “design out” negative properties without getting caught in a loop of regrettable substitutions.

5. Conclusions

In this study, we combined *in vitro* bioassays with high resolution non-target mass spectrometry to characterize the toxicity and chemical composition of bio-based and biodegradable materials. Our results indicate that the majority (67%) of bioplastics and plant-based products contain toxic chemicals as well as a large number and diversity of compounds (> 1000 chemical features each in 80% of the samples). Importantly, we applied solvent extraction in order to analyze the intrinsic chemical toxicity present in the products. In future work, migration studies with food simulants are needed in order to identify the toxicity and chemicals migrating under real-world conditions and to estimate the human exposure to those.

Our study demonstrates that bio-based and/or biodegradable materials available on the market are just as toxic as conventional plastics with regards to the chemicals they contain. This highlights that the positive connotation of “biological” or “sustainable” materials does not extend to chemical hazards. Accordingly, our findings imply that in order to develop bio-based/biodegradable materials that indeed outperform conventional plastics, sustainability and chemical safety aspects must be addressed alike. One way to promote this is to integrate chemical toxicity into the life cycle assessment of materials.

On a positive note, we show that safer products are already at the market that can be used as best practice examples. Additionally, the chemical safety of materials can be further optimized using green chemistry to “design out” toxicity during the development of new bio-based and biodegradable materials. Besides these human health aspects, the carbon, energy, water and land footprints need to be minimized to create truly better plastics or plastic alternatives and avoid regrettable substitutions.

CRediT authorship contribution statement

Lisa Zimmermann: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Andrea Dombrowski:** Investigation, Writing - review & editing. **Carolin Völker:** Writing - review & editing, Supervision, Funding acquisition. **Martin Wagner:** Conceptualization, Methodology, Formal analysis, Visualization, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106066.

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A3 Plastic products leach chemicals that induce *in vitro* toxicity under realistic use conditions

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Declaration of author contributions to the publication:

	LZ	JO	KB	ZB	CV	MW
Concept and design	**70%**	–	–	–	10%	20%
Conducting tests and experiments	**35%**	–	55%	10%	–	–
Compilation of data sets and figures	**80%**	–	–	–	–	20%
Analysis and interpretation of data	**70%**	–	–	–	–	30%
Drafting of manuscript	**60%**	5%	1%	4%	5%	25%

Plastic products leach chemicals that induce *in vitro* toxicity under realistic use conditions

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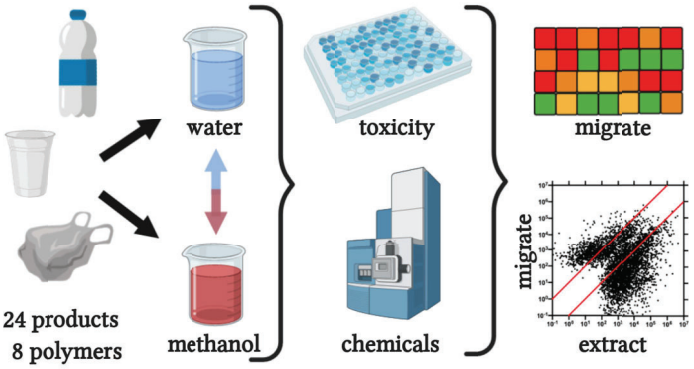
ABSTRACT

Plastic products contain complex mixtures of extractable chemicals that can be toxic. However, humans and wildlife will only be exposed to plastic chemicals that are released under realistic conditions. Thus, we investigated the toxicological and chemical profiles leaching into water from 24 everyday plastic products covering eight polymer types. We performed migration experiments over 10 d at 40 °C and analyzed the migrates using four *in vitro* bioassays and nontarget high-resolution mass spectrometry. All migrates induced baseline toxicity, 22 an oxidative stress response, 13 antiandrogenicity and one estrogenicity. Overall, between 17 and 8936 relevant chemical features were present in the migrates. Compared to all plastic chemicals detected in a product, this corresponds to 1 and 84%. Further, we tentatively identified ~3% of all detected features implying that most plastic chemicals remain unknown. The toxicity and the number of compounds were specific to the product rather than the polymer type indicating that a generalization based on the material is not possible. Our results demonstrate that plastic products readily leach many more chemicals than previously known, some of which are toxic *in vitro*. This questions whether the chemicals migrating from plastic are safe.

ABSTRACT ART



1 INTRODUCTION

Individual plastic chemicals, as bisphenol A and phthalates have received much scientific and public attention. However, plastics are not composed of single compounds but contain a wide variety of chemicals:[1] more than 4000 chemicals have been associated with plastic packaging alone.[2] These include starting substances such as monomers, oligomers and polymers as well as additives, including plasticizers, antioxidants, heat stabilizers and pigments. In addition, plastics contain an unknown number of non-intentionally added substances (NIAS), that is, impurities of the starting substances and additives as well as intermediates, reaction and breakdown products formed during processing.[3] The total number of plastic chemicals, consisting of intentionally and non-intentionally added substances, is unknown as is their mixture toxicity. Thus, we extracted everyday plastics with methanol in our previous study and demonstrated that they contain complex chemical mixtures that induce *in vitro* toxicity.[4]

Since most plastic chemicals are not covalently bound to the polymer matrix, they can leach into the packaged goods in case of packaging. In the context of human health, such chemical migration is especially relevant for food contact materials (FCMs) as compounds leaching into foodstuff will become available for human exposure. Plastic chemicals can also leach into natural environments in the case of littering resulting in the exposure of wildlife. Previous studies have demonstrated that the chemicals migrating into aqueous media include organic compounds and metals,[5] phenols and phthalates[6, 7] as well as known estrogenic chemicals.[8] However, concerns have been raised regarding the lack of hazard information for chemicals known to be present in FCMs, including plastics, as well as the challenge of unknown compounds migrating from such materials.[9]

One approach to tackle the chemical complexity of plastics, including the large number of unknown chemicals and mixture effects, is whole migrate toxicity testing.[10] Indeed, *in vitro* bioassays have been applied already to determine the overall toxicity of the chemical mixtures leaching from plastics.[11] Here, plastic migrates induced unspecific effects in *Aliivibrio fischeri*[12] and *Photobacterium phosphoreum,*[13] cytotoxicity as well as endocrine activity.[14, 8, 15] However, a comprehensive comparison of the extractable chemicals present in plastics and the compounds leaching under more realistic conditions including their toxicity is missing.

Thus, we selected 24 plastic products covering eight polymer types, performed migration experiments with water and analyzed these migrates for baseline toxicity, oxidative stress induction and endocrine activity. Subsequently, we compared the *in vitro* effects with those induced by methanolic extracts of the same samples.[4] In addition, we performed nontarget

high-resolution mass spectrometry (UPLC-QTOF-MS^{E}) to characterize and compare the extractable and leachable chemicals. Accordingly, our results shed light onto the fraction of plastic chemicals and their toxicity available for human and wildlife exposure.

2 MATERIAL & METHODS

2.1 Sample selection

We selected 24 commonly used plastic products (Table 1) covering eight polymer types (HDPE, LDPE, PS, PP, PET, PVC, PUR and PLA) that induced *in vitro* toxicity in our previous study (HDPE 1 corresponds to HDPE 3, PP 1 to PP 2, PP 2 to PP 3 and PET 1 to PET 3).[4] Besides all active products (but PP 5 as it was removed from assortment), we included LDPE 3 as representative of non-toxic products. Half of the 24 products were food contact materials (FCMs). We purchased the products in local retailer stores and confirmed their polymer types using Fourier-transform infrared spectroscopy (FTIR, PerkinElmer, Spectrum Two, Waltham, Massachusetts) in our previous study. The spectra of the samples are available under DOI: 10.5281/zenodo.3263830.

2.2 Migration experiment

To avoid sample contamination, we used glass or polytetrafluoroethylene (PTFE) consumables whenever feasible, rinsed all materials twice with acetone (pico-grade, LGC Standards) and annealed glass items at 200 °C for ≥ 3 h. Additionally, we conducted the sample preparation and the bioassays under a laminar flow hood. For sample preparation, the content was removed from packaging samples and the products were rinsed thoroughly with ultrapure water until all residues were removed. All samples were cut into 0.5–1.5 × 2 cm pieces. Foamy products were cut to a thickness of 0.5 cm.

Based on the results of an initial experiment (see SI for details), migration conditions were set to 10 days (d) at 40 °C and 60% relative humidity in the dark which corresponds to the migration testing conditions laid out in the EU regulation for plastic food contact materials.[16] 60.8 g were leached in 1520 mL ultrapure water (exception PET 1: 30.8 g in 760 mL), corresponding to 40 mg plastic mL^{-1} water. After 10 d, the solution was filtered through porcelain funnels into new 2-L glass bottles. Foamy samples were additionally squeezed using syringes to recover most of the water. The recovered volume of ultrapure water was determined, and 20 mL were transferred into new brown glass vials and stored at 8 °C (aqueous migrates). The remaining sample was extracted using solid phase extraction (SPE; migrates). To contextualize the bioassay results, we use plastic equivalents in such that “1 mg plastic” represents the toxicity migrating from 1 mg plastic per well in the respective bioassays.

2.3 Solid Phase Extraction

We used C18-silica gel cartridges (TELOS C18(EC)/ENV, 700 mg, 6 mL, 697-70M-006Z, Kinesis, Wertheim, Germany) to extract the aqueous samples. SPE columns were sequentially conditioned with 2 mL n-heptane (Carl Roth, CAS: 142-82-5, purity ≥ 99.9%) followed by 2 mL acetone (Carl Roth, CAS: 67-64-1, ≥ 99.9%), 6 mL methanol (Carl Roth, CAS: 67-56-1, ≥ 99.95%) and 8 mL ultrapure water by gravity. The pH of the aqueous samples was adjusted to 2.5 using 3.5 M sulfuric acid (VWR, CAS: 7664-93-9, 96%) before loaded on the columns with a constant vacuum flow of approximately 2–5 mL min^{-1}. The cartridges were dried under liquid nitrogen and eluted with 5 mL acetone followed by 5 mL methanol. The combined extracts were evaporated to dryness under nitrogen and re-dissolved in approximately 150 µL dimethyl sulfoxide (DMSO, Carl Roth, CAS: 67-68-5, ≥ 99.9%). The volume of DMSO was adjusted to the volume of each sample to generate extracts that are 10,000-times concentrated and equivalent to 400 mg plastic $µL^{-1}$ DMSO. These migrates were stored in glass vials with PTFE caps at -20 °C prior to analysis.

Six procedural blanks (PB 1–6, two per run) consisting of glass bottles not containing any sample but ultrapure water only as well as three SPE blanks (SPE 1–3, one per run) consisting of 1.5 L ultrapure water directly applied to SPE were treated identically to control for a potential contamination.

2.4 Bioassays

All bioassays were conducted in 96-well microtiter plates with negative controls, solvent controls (DMSO for migrates only), PB 1–6 and SPE 1–3. Aqueous migrates, solvent controls and blanks in ultrapure water were diluted 1.4-fold (baseline toxicity) and 1.6-fold (endocrine activity). Migrates in DMSO were diluted 100-fold (baseline toxicity), 200-fold (oxidative stress response) or 480-fold (endocrine activity) with medium, resulting in a maximum final solvent concentrations of 1, 0.5 or 0.2% (v/v), respectively. Throughout the main experiments, none of the blanks induced toxicity (Figure S1 and S2). Thus, there was no contamination during the migration, extraction and analysis and pooled blanks (control, C) are presented in bioassay results (Figure S4–S8).

Baseline toxicity. The Microtox assay with the bioluminescent bacterium *Allivibrio fischeri* was performed according to an ISO guideline[17] as described previously[4] with minor modification for testing aqueous migrates. These were adjusted to a conductivity of 25–45 mS cm^{-1} by addition of sodium chloride. 50 µL of *A. fischeri* suspension was added to 125 µL of the aqueous migrate. Negative and positive controls (3,5-dichlorophenol, Table S3, Figure

S3) and migrates were analyzed in 1:2 serial dilutions corresponding to concentrations of 39.1 µg – 5.0 mg and 18.31 µg – 600 mg plastic well^{-1} (PVC: 71.53 ng – 600 mg) for aqueous migrates and migrates, respectively. Results from three to six independent experiments (dots in graph), each with two technical replicates, were expressed as effect concentration (EC_{20}, EC_{50} ± SEM, mass of plastic well^{-1} inducing a 20%, 50% luminescence inhibition) and mean effect size ± SEM (luminescence inhibition induced by 22.5 mg plastic well^{-1}) if in $n \geq 1$ 20% or 50% inhibition was reached, respectively. In case an EC_{20} or EC_{50} could not be derived, we used an EC of 6.25 mg plastic well^{-1} for aqueous migrates and 750 mg plastic well^{-1} for migrates to visualize the data, indicating that the EC is larger than the highest analyzed concentration (HAC).

Oxidative stress response. We used the AREc32 assay to investigate the induction of an oxidative stress response in the Nrf2/ARE pathway.[18] The AREc32 assay as well as the determination of cell viability were performed as described elsewhere.[19] We analyzed eight concentrations of the migrates in serial dilutions (1:2, 1.56–200 mg plastic well^{-1}) and the reference compound *tert*-butylhydroquinone (t-BHT, Table S3, Figure S3). Each sample was analyzed in three independent experiments (dots in graph) with duplicates each. We excluded the concentrations that were cytotoxic in the respective experiment and replicate before deriving induction ratios (IR) as well as the effect concentration producing an IR of 2 over the control (EC_{IR2}). In case an EC_{IR2} could not be derived, we used an EC_{IR2} of 250 mg plastic to visualize the data, indicating that the EC_{IR2} is larger than the HAC.

Endocrine activity. We used yeast-based reporter-gene assays to investigate the induction of agonistic activity at the human estrogen receptor α (hERα)[20] and antagonistic activity at the human androgen receptor (hAR).[21] The Yeast Estrogen Screen (YES) and the Yeast Antiandrogen Screen (YAAS) with the reference compounds, 17β-estradiol and flutamide (Table S3, Figure S3), respectively, were performed as previously described with minor modifications.[4] Samples were analyzed in concentrations of 3.0 mg (aqueous migrates) or 0.2–100 mg plastic well^{-1} (migrates) and in two to four independent experiments with eight replicates, each. Cytotoxic migrates were analyzed in 1:2 serial dilutions down to 9.9 ng plastic well^{-}1 (PLA 3) in the YES and 0.02 ng plastic (PP 3), 9.90 ng plastic (PLA 3) as well as 0.38 µg plastic well^{-1} (PLA 4) in the YAAS assay. The limit of detection (LOD) of each experiment was calculated as three times the standard deviation (SD) of pooled negative and solvent controls. Mean effects > LOD were considered significant. Plastic equivalents inducing 20% cytotoxicity (EC_{20}) as well as 50% relative endocrine activity (EC_{50}, calculated if $n \geq 1$ had a relative activity > 50%) are reported. In case an EC_{50} could not be derived, we

used an EC_{50} of 3.75 mg for aqueous migrates and 125 mg plastic well^{-1} for migrates indicating that the EC_{50} is larger than the HAC. To ensure comparability, only those experiments were considered in which the concentration-response relationship of the reference compound had a $r^2 > 0.9$, a minimal relative luminescence unit < 5,000 and a maximal > 50,000 as well as an EC_{50} next to 8×10^{-11} mol L^{-1} 17β-estradiol (YES) or 1.5×10^{-5} mol L^{-1} flutamide (YAAS, Table S3).

Analysis of bioassay data. We used GraphPad Prism 5 and 8 (GraphPad Software, San Diego, CA) for nonlinear regressions (four-parameter logistic models) and statistical analyses. In order to present toxicities of plastic migrates in a heat map (Figure 1), *in vitro* data were plotted as gradient from 0 (green) to 100% (red) toxicity. The endocrine activities were used as such. Effects in the Microtox and AREc32 assay were normalized to the lowest and highest effect observed for the respective endpoint. For AREc32, effect levels (ELs) and endocrine activities of the highest non-cytotoxic concentrations (Table S5, S6) were used. For the comparison of extracts[4] and migrates (Figure 2), ECs were set to the HAC and endocrine activities to zero in case the sample did not induce an effect. If cytotoxicity occurred, the highest concentration that was nontoxic for both, extract and migrate, was compared (antiandrogenic activity: PVC 2: 0.78 mg; estrogenic activity: PVC 2 and PLA 1: 0.94, PS 2: 0.47, PLA 3: 0.03 mg plastic well^{-1}).

2.5 Chemical analysis

Nontarget screening was conducted using an ultra-high performance liquid chromatograph AQUITY I-Class UPLC coupled to a hybrid quadrupole orthogonal time-of-flight mass spectrometer SYNAPT G2-S HDMS (both Waters, Milford, MA, USA). The UPLC system was equipped with a binary pump, an online vacuum degasser, an autosampler, and a thermostated column compartment. The separation was carried out on an Acquity UPLC BEH C18 column (130 Å, 1.7 µm, 2.1 × 150 mm) equipped with a guard column C18 (both Waters), with mobile phases (A) H_2O and (B) methanol, both with 0.1% formic acid. The gradient of B was set as follows: 0 min, 20%; 0.5 min, 20%; 4.5 min, 60% (6); 35.5 min, 100% (6); 38.5 min, 100%; 39.5 min, 20% (6); 41.5 min, 20%. The column temperature was maintained at 40 °C, the flow rate was set to 0.2 mL min^{-1} and the injection volume was 2 µL.

The mass spectrometer was equipped with an ESI source operated in positive mode. The MS detection conditions were set as follows: capillary voltage 2.5 kV, cone voltage 30, source offset voltage 60 V, source temperature 120 °C, desolvation temperature 350 °C, desolvation gas flow 800 L h^{-1}, cone gas flow 100 L h^{-1}, collision gas flow 0.15 mL min^{-1},

nebulizer gas pressure 6 bar. The mass spectrometer was operated in data-independent MS^E acquisition mode and a high collision energy ramped from 15 to 45 eV. Data were acquired from 2 to 35 min over the mass range of 50–1200 Da and the resolution of the mass spectrometer was 20,000.

Prior to analysis, the migrates as well as the extracts of our previous study[4] were diluted in 1:1 methanol:water (v:v) to yield a concentration of 0.24 mg plastic μl^{-1}. Each sample was analyzed once. Quality controls (QCs) were prepared by pooling aliquots of each individual sample, one QC was prepared for the extracts and another one for the migrates. LC blanks (1:1 methanol:water) and QCs were injected regularly after 10 sample injections to check for contamination and monitor performance of the instrument. The mass spectral data of all samples can be accessed under DOI: (to be added after publication).

Data analysis and compound identification. We used Progenesis QI (version 2.3, Nonlinear Dynamics) to analyze the UPLC-QTOF-MS/MS data. In brief, we imported the raw data files of six PBs for the migrates, three PBs for the extracts and of the extracts and migrates of each plastic sample individually. The lock-mass correction with leucine enkephalin was done online. We enabled the search for common adducts (M+H, M+2H, M+H-H_2O, M+H-2H_2O, M+Na, M+2Na, M+H+Na, M+2Na-H, M+NH_4, M+CH_3OH+H, M+K, M+ACN+H, M+ACN+Na, 2M+H, 2M+Na, 2M+ACN+H, 2M+ACN+Na), automatically aligned the retention times of all runs, and performed the peak picking (automatic sensitivity, no predefined peak width, retention times < 2 min excluded, fragment sensitivity of 0.2 % of the base peak).

We exported the resulting feature list to Microsoft Excel for Mac (version 16.35) and compared the maximum raw abundance of each feature in the PBs (n = 9) to the raw abundance of the same feature in the extract or migrate of the respective sample. We filtered for features that were not present in PBs but in the sample or had a tenfold higher abundance in the sample than in the PBs. The resulting feature list represents all chemicals detected in either the extract, the migrate or both. We determined the ratio of the raw abundance of each feature in the migrate and the extract to determine how many features did not migrate (ratio < 0.1), migrate (ratio > 0.1), or were newly formed in water (not present in extracts). The migration cut-off is based on the assumption that if a compound has less the 10% abundance in the migrate compared to the extract, migration would be low. While this represents a pragmatic approach, the concentration of chemicals classified as “not migrating” might nonetheless be significant.

We tentatively identified all features detected in the samples using the Metascope algorithm in Progenesis QI for *in silico* fragmentation. In brief, we constructed three databases (see SI for details) covering the chemicals present in plastic packaging (CPPdb, 2680 compounds),[2] the chemicals registered under the REACH regulation in 2020 (ECHAdb, 7092 compounds),[22] and the chemicals (pre)registered under REACH in 2017 as provided by the NORMAN Suspect List Exchange (NORMANdb, 65,738 compounds).[23] These databases were queried individually for each sample with a precursor tolerance of 5 ppm and a fragment tolerance of 10 ppm. The results of the tentative identification were filtered for hits with a score > 40 (based on fragmentation, mass and isotope similarity, max. 60). If a feature had multiple identification with a score > 40, the one with the highest score was picked. The results of the identification with the three databases were combined and duplicates removed retaining the identification with the highest score per feature.

3 RESULTS & DISCUSSION

In our previous study, we demonstrated that consumer plastics contain extractable chemicals inducing *in vitro* toxicity. Since exposure only occurs if these extractable compounds also leach under realistic conditions, we performed migration experiments with water using the conditions set out by the European Union regulation of food contact materials.[16] It is assumed that the toxicity of the migrate can serve as indicator for the chemical toxicity readily released from the plastic product in conditions commonly encountered during use or after disposal (e.g., migration into packed foodstuff, leaching in aquatic environments).

3.1 Plastic products leach toxicity

All plastic products we investigated leached chemicals triggering *in vitro* toxicity (Figure 1).

Baseline toxicity. Each sample induced baseline toxicity with the PVC migrates (1, 2 and 3) being most potent (EC_{50} < 5 mg plastic well^{-1}, Table S4, Figure S4). The widespread induction of baseline toxicity is in accordance with previous research,[24] and shows that migrating plastic chemicals trigger unspecific toxicity. The fact that all samples were active in the Microtox assays is probably related to our sample selection (based on the toxicity of the extracts) and the fact that a broad range of compounds causes baseline toxicity.[25]

Oxidative stress response. In addition, all samples except HDPE 1 and PLA 4 activated the Nrf2-ARE regulated oxidative stress response (Table S5, Figure S5). Here, LDPE 4 was most potent (EC_{IR2} = 2.15 mg plastic well^{-1}) and PS 1 had the highest effect level (IR = 80). A widespread release of chemicals inducing an oxidative stress response from plastic products has not been reported in the literature, so far. However, the leachates of UV weathered PE, PET, PP, and PS microplastics were more active in the AREc32 assays than dark controls indicating that polymer degradation products contribute to the oxidative stress induction.[26]

Endocrine activity. PVC 2 was the only sample that leached estrogen receptor agonists above the LOD (2.3%) with a relative activity of 59.4% at 1.56 mg plastic well^{-1} and an EC_{50} of 0.27 mg plastic well^{-1} (Table S6, Figure S6A). The chemicals migrating from PVC 2 also induced the strongest antiandrogenic effects (EC_{50} = 0.28 mg plastic well^{-1}). In total, 13 samples inhibited the androgen receptor above the LOD (27.3%, Table S6, Figure S7A). PUR 4, HDPE 1, LDPE 4, and PVC 2 had an antiandrogenicity > 90% at 100 mg plastic well^{-1}. This is in line with a number of studies that demonstrate the leaching of estrogenic or antiandrogenic compounds from multiple types of products and polymers.[8, 15, 27–29] Interestingly, our results show that the migrates' antiandrogenicity was more pronounced and

potent than their estrogenicity. This has been reported before for PP, PE and PS FCMs.[15] Importantly, the hypothesis that a stronger antiandrogenicity might be specific to yeast-based reporter-gene assays needs to be verified in future research.

We assessed the toxicity of migrates up to relatively high concentrations, covering the maximum equivalent of chemicals migrating from 100 mg (endocrine activity), 200 mg (oxidative stress), and 600 mg plastic well^{-1} (baseline toxicity). Nonetheless, many samples were very potent (EC_{20}s well below 10 mg plastic well^{-1}) and induced toxicity at low concentrations. As an example, the chemicals migrating from < 0.3 mg PVC 2 induced 50% estrogenicity (Figure S6A) and antiandrogenicity (Figure S7A). Given that the mass of plastic products we use on a daily basis is much higher, this implies that human exposure to the chemicals leaching from plastics is not negligible.

3.2 The toxicological signature is product-specific

A comparison of the toxicological signatures of the migrates highlights that the toxicity migrating from plastics is specific to the product rather than the polymer type. Consistent with our previous results,[4] the compounds migrating from PVC and PUR samples were most toxic. For instance, PVC 2 affected all endpoints with a high potency. Eleven samples induced toxicity on three out of the four endpoints. These include all PUR migrates, three out of four LDPE migrates and at least one sample of every other polymer type (exception: HDPE, PET with only one tested product). However, the levels of toxicity varied within all polymer type categories. As an example, the toxicity migrating from PS 3 and PLA 4 was much lower than the one observed for other samples made of the same polymers. This supports our notion that the chemical safety of plastic products cannot be generalized based on their polymer type.

Safety is of particular importance for products with food contact. Thus, we compared the toxicity of products intended for food contact (12 FCMs) with those not intended for food contact (12 non-FCMs, Table 1). Interestingly, both groups had a comparable potential to induce baseline toxicity and oxidative stress (Figure 1). More non-FCMs than FCMs induced antiandrogenicity but individual FCMs also released antiandrogenic compounds (e.g., LDPE 3, PS 1, PVC 1). This underpins concerns over the adequacy of the traditional approach of assessing the safety of FCMs that prescribes to assess the migration of starting substances.[16] Concurrently, our results supports calls for whole migrate toxicity testing of the marketed products.[9]

3.3 The *in vitro* toxicity of migrates and extracts is not identical

To investigate whether the toxicity present in plastics leaches to water, we compared the effects of methanolic extracts and migrates using the identical concentration ranges (Figure 2, Table S7). For the former, we used the data from our previous study that was generated using the same samples and bioassays.[4] The chemicals present in and leaching from eleven products induced a similar, high baseline toxicity (Figure 2A), including all PVC, three out of four LDPE as well as one PP, PS and PLA products, each. Three PLA and two PUR products contained chemicals that inhibited bioluminescence but these did not migrate into water. In contrast, two products (PP 1, PUR 4) leached a higher baseline toxicity in water than methanol.

The chemicals activating an oxidative stress response were more readily extractable than leachable (Figure 2B). Here, the extracts and the migrates of LDPE 4, PP 1 and PVC 1 induced a similar toxicity. Ten other extracts activated this pathway with high efficiency but related migrates did not induce oxidative stress. With regards to endocrine effects, the estrogenicity detected in the extract of PVC 2 readily migrated into water (Figure 2C). Here, the estrogenicity of the migrate (60.3% at 3.75 mg $well^{-1}$) was stronger than that of the extract (27.1%). The picture was similar for the antiandrogenicity of this sample (migrate: 90.9% vs. extract: 40.1%, Figure 2D). Eight other products contained antiandrogenic chemicals $>LOD_{extract}$ (29.2%) that did not leach into water.

As expected, these results show that not all *in vitro* toxicity detected in plastic extracts is migrating into water. This may be due to the fact that not all extractable chemicals are leaching and/or that the concentration of the leachable chemicals is lower than that of the extractable ones. Interestingly, chemicals inducing baseline toxicity had a higher migration potential than those triggering oxidative stress or antiandrogenic activity. Again, this might be related to the large number of compounds triggering baseline toxicity. In case of migrate samples that induced a higher toxicity than their extract counterpart did (PET 1, PUR 4), the causative compounds may dissolve better in water than in methanol. In addition, degradation products of the leaching compounds (e.g., by hydrolysis) might add to the toxicity. Both might also be true for the chemicals inducing endocrine activity in the migrate of PVC 2.

3.4 SPE extracts the toxicity from aqueous migrates but needs improvement

In order to assess the efficiency of the SPE to extract toxicity from the migrates, we also assessed the baseline toxicity (Figure S8) as well as the estrogenic (Figure S6B) and antiandrogenic activity (Figure S7B) of aqueous migrates (without SPE). When comparing

the concentration-response relationships for baseline toxicity with migrates (with SPE), both sample types induced rather similar effects (Figure S9). However, for some samples (PS 1, PET 1, PLA 3), the baseline toxicity was higher in the aqueous migrates than in the extracted migrates (Table S8). With regards to the antiandrogenic activity, the aqueous migrate of one sample (PP 1, Table S8) induced an effect whereas the corresponding migrate produced via SPE did not. This indicates that the compounds inducing toxicity were not recovered completely by the SPE method, similar to what has been observed for drinking water and wastewater.[30, 31] Accordingly, the sample preparation of aqueous media used for migration testing must be optimized to recover the maximum *in vitro* effects for future whole migrate toxicity testing.

3.5 Several thousand chemicals migrate from plastics

We detected between 685 (PS 3) and 17,973 (PUR 3) unique chemical features in the extracts and migrates of the 24 plastic products altogether (Table 2). Out of these, 296–4294 features were only detected in the extracts, that is, they were not migrating from the plastic products. Between 613 and 10,766 features (low migration) were present in the extracts and migrates with an at least 10-fold higher abundance in the former compared to the latter (ratio of < 0.1). Thus, we classified these features as having a minor migration potential. In contrast, 17 (PS 4) to 8572 features (PUR 3) were readily leachable, that is, they were detected in the migrate with an abundance of at least 10% compared to the respective extract (ratio of > 0.1). In addition, up to 1612 features were only detected in migrates but not in extracts. That implies that these have been newly formed in water or are not extractable with methanol. In total, we found that between 17 (PS 4) and 8936 (PUR 3) features were either readily migrating from the plastic products or newly formed in the migrates. In half of the migrates, we detected more than 2000 chemical features. Thus and in contrast to other studies using nontarget chemical analysis,[32, 33] we show that many more chemicals are migrating from plastic products than previously known. Importantly, our approach is conservative and rather underestimates the number of migrating chemicals because (1) the concentration of the analyzed migrates was rather low (chemicals migrating from 0.48 mg plastic), (2) the extraction via SPE probably does not recover 100% of the compounds, and (3) we only used positive ionization in the mass spectrometry.

Some of the plastic products leached very few chemicals (PLA 4 < PLA 2 < PS 4 < PET 3 < PLA 1 < LDPE 2, Figure 3A). In these samples, less than 8% of all detected features were readily leachable or newly formed in the migrates. On the other end of the spectrum, more

than one third of all features detected in a sample leached from PVC 1, PS 2, PP 2, PLA 3, PUR 3, PUR 1, PS 1, LDPE 4, and PUR 2. In the latter sample, 83.8% of all features (7875 out of 9402) were present in water after 10 d of migration. As in our previous work analyzing extracts,[4] there was no clear association of the number of migrating compounds with the polymer type: Products made of PE, PS, PET, and PLA leached relatively few chemicals while those made of PP, PVC and PUR leached many. However, there were notable exceptions, including LDPE 4, PS 2, and PLA 3 (many features in the migrates), as well as PVC 3 and PUR 4 (few features), making it impossible to generalize.

Taking the abundance of a feature as a proxy of its quantity, many of the migrating compounds are detected in similar levels in the extracts and the migrates, indicating they are readily leachable in water (Figure 3B, Figure S10). The abundance of many migrating features falls in a band of 10-fold higher to 10-fold lower than in the extracts (e.g., in LDPE 4, PVC 1, PVC 4, PUR 2, PUR 3). However, there were also several features that we detected in much higher levels in the migrates than in the extracts, no matter the polymer type (e.g., in LDPE 3, PP 2, PUR 1, PLA 3). This implies a preferential migration into water over methanol or an additional formation during migration. Nonetheless, these results have to be interpreted with caution given that the abundance of a feature in the mass spectroscopy may not be linearly related to its concentration.[34]

3.6 Most plastic chemicals remain unknown

By *in silico* fragmenting the compounds in the databases of Chemicals associated with Plastic Packaging (CPPdb), chemicals registered under REACH (ECHAdb), and the NORMAN Suspect List Exchange (NORMANdb), we tentatively identified 2912 unique compounds present in and/or migrating from the plastic products. This represents approximately 3% of all detected features. Most of the chemicals were identified using the NORMANdb (2491 compounds). The CPPdb had the best coverage with 7.3% of the compounds in that database being detected in plastic products. Interestingly, only 225 chemicals were covered by the ECHAdb, that is, they are registered under the European REACH regulation.

In each individual sample, we identified between 1 (PS 3, PS 4) and 822 (PVC 1) chemicals (Table 2). Generally, the identification rates (number of tentatively identified compounds out of all features detected in a sample) we achieved were very low, ranging from almost 0% in PLA 2 to 9.6% in LDPE 4. This demonstrates that most plastic chemicals remain unknown. This is even more true given that our *in silico* approach may result in many false-positive identifications.

The ten most frequently identified chemicals across all plastic products include the cyclic compounds cyclooctane-1,2,3,4-tetrol, cyclooctane-1,2,3-triol and 1,4,7,10,13,16,19-heptaoxacyclohenicosane as well as multiple ethylene glycols (Table S9). Interestingly, all compounds were identified multiple times in the same sample, indicating the presence of isomers. We prioritized the ten features with the highest abundance in each plastic migrate. Out of a total of 240 features, we tentatively identified 46 chemicals, including multiple carboxylic acids, alcohols, and amides (Table S10). Interestingly, the organophosphates migrating from PVC products were the only tentatively identified chemicals that are obviously related to plastic additives. In these cases, the detected compounds are probably degradation products of the flame retardant tris(2-butoxyethyl) phosphate (TBEP, migrating from PVC 4). In addition to these plastic additives, another seven compounds are associated with plastic packaging according to Groh et al. (2019)[2], including 2-[2-[2-(2-methylprop-2-enoyloxy)ethoxy]ethoxy]ethyl 2-methylprop-2-enoate (triethylene glycol dimethacrylate used as lubricant), [2-hydroxy-3-[4-[2-[4-[2-hydroxy-3-(2-methylprop-2-enoyloxy)propoxy]phenyl]propan-2-yl]phenoxy]propyl] 2-methylprop-2-enoate (bisphenol A glycidylmethacrylate used as filler and adhesive), 2-[2,2-bis(2-prop-2-enoyloxyethoxymethyl)butoxy]ethyl prop-2-enoate (ethoxylated trimethylolpropane triacrylate used as colorant and adhesive), 2-[2,2-bis(2-prop-2-enoyloxyethoxymethyl)butoxy]ethyl prop-2-enoate (ethoxylated trimethylolpropane triacrylate used as solvent, filler, colorant, and adhesive), 2-benzyl-2-(dimethylamino)-1-(4-morpholin-4-ylphenyl)butan-1-one (Irgacure 369 used as UV photoinitiator), and 2-(dimethylamino)-2-[(4-methylphenyl)methyl]-1-(4-morpholin-4-ylphenyl)butan-1-one (photoinitiator 379). Although only tentative based *in silico* fragmentation, the identification of these compounds appears plausible, especially for those covered by the CPPdb. Importantly and in a more general context, the compounds we tentatively identified here do not belong to the "usual suspects", such as bisphenols and phthalates. Accordingly, we assume that very little – if any – (eco)toxicological information is available regarding the chemicals actually leaching from plastics. That is to say, the hazards of plastic chemicals humans and wildlife are likely exposed to remain largely unknown and, thus, unregulated.

Our study highlights that plastic products leach chemicals triggering toxicity. While the prevalent baseline toxicity points towards unspecific effects relevant in an environmental context, the prevalent antiandrogenicity is an indicator for the leaching of endocrine disrupting chemicals relevant for human health. Our results also show that many more chemicals are migrating from plastics than previously known. The large number of

compounds, and the fact that most of these remain unidentified, pinpoints the shortcomings of current regulatory approaches to the chemicals leaching from plastics. As an example, very few of the chemicals migrating from plastic products marketed in the European Union are covered by REACH. Accordingly, we do not know whether the chemicals leaching from consumer plastics are safe. The combination of whole migrate toxicity testing and nontarget chemical analysis used in this study represent a good option to address these regulatory gaps. While an assessment of the human health implications is beyond the scope of our study, the sheer number of leaching plastic chemicals calls for intensified research, regulatory attention as well as for action to improve the chemical safety of plastics. Regarding the latter, the chemical composition of plastics can be simplified by reducing the number of starting substances and additives and by better controlling polymerization and processing. Another approach would be to keep plastics chemically complex but significantly reduce the migration by covalently binding additives to the polymer backbone, reducing the diffusion coefficient of the polymer or introducing additional barrier functions. In any case, such improvements require a fundamental rethinking and redesign of the plastics we are using today.

ASSOCIATED CONTENT

Supporting Information

Additional material and additional figures and tables: Methodology and results to determine migration conditions, methodology chemical analysis, information on databases used for compound identification, further *in vitro* toxicity data of migrates and aqueous migrates (baseline toxicity, oxidative stress, estrogenicity, antiandrogenicity), of reference compounds and samples, toxicity of migrates vs. non-extracts, toxicity of migrates (with SPE) vs. aqueous migrates (without SPE), tentatively identified compounds (most frequent across all samples, with highest abundances), abundance of chemical features detected in migrates vs. extracts.

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Author contributions

CV acquired funding and administered the project, LZ, CV and MW conceived the study, LZ and KB performed the bioassay experiments, LZ and ZB performed the chemical analyses, LZ and MW analyzed the data, LZ and MW wrote the manuscript, all authors provided comments on the manuscript.

Notes

The authors declare no competing financial interest. For the sake of transparency, we include the following information: MW is unremunerated member of the scientific advisory board of the Food Packaging Forum, a non-profit foundation making scientific facts and expert opinions about food packaging and human health accessible and understandable to all its stakeholders. He does not receive any personal benefit from this work, financially or otherwise.

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TABLES & FIGURES

Table 1. Plastic products analyzed in this study. FCM: food contact material.

Sample	Plastic product	FCM
HDPE 1	Bin liners	
LDPE 1	Lemon juice bottle	+
LDPE 2	Plastic wrap	+
LDPE 3	Freezer bag	+
LDPE 4	Hair conditioner bottle	
PS 1	Yogurt cup	+
PS 2	Fruit tray	+
PS 3	Vegetable tray	+
PS 4	Plastic cup	+
PP 1	Yogurt cup	+
PP 2	Gummi candy packaging	+
PET 1	Oven bag	+
PVC 1	Plastic wrap	+
PVC 2	Place mat	
PVC 3	Pond liner	
PVC 4	Floor covering	
PUR 1	Scouring pad	
PUR 2	Kids bath sponge	
PUR 3	Acoustic foam	
PUR 4	Shower slippers	
PLA 1	Yogurt cup	+
PLA 2	Vegetable tray	+
PLA 3	Shampoo bottle	
PLA 4	Coffee cup lid	+

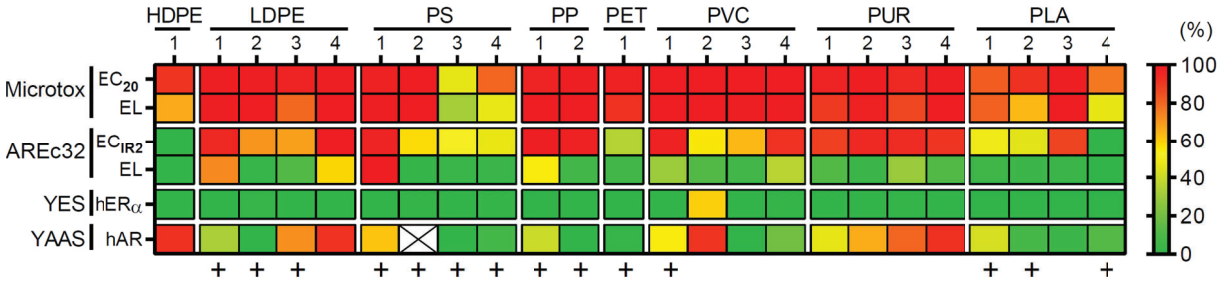

Figure 1: *In vitro* toxicity of chemicals migrating from plastic consumer products. Baseline toxicity (Microtox) and oxidative stress response (AREc32) are presented as effect concentrations inducing 20% baseline toxicity (EC_{20}) or an induction ratio of 2 (EC_{IR2}) as well as effect levels (EL) at the highest analyzed noncytotoxic concentration. Estrogenic (YES) and antiandrogenic activities (YAAS) are shown as relative (%) activation of the human estrogen receptor α (hERα) and inhibition of the androgen receptor (hAR). Note: x, all analyzed concentrations were cytotoxic; +, food contact materials.

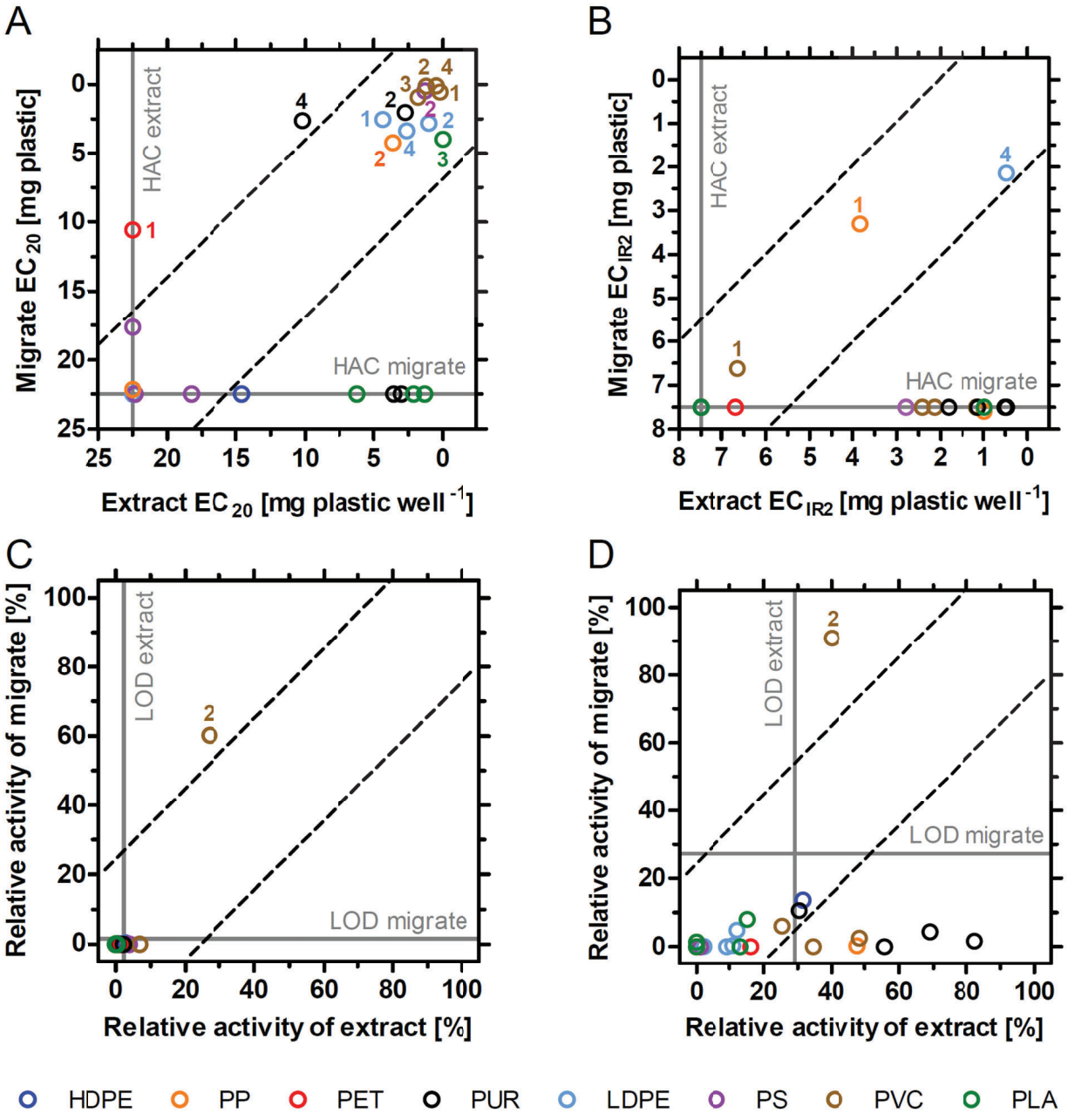

Figure 2. Comparison of *in vitro* toxicity present in plastics (extracts) and leaching from plastic (migrates). For baseline toxicity (*A*) and oxidative stress induction (*B*), effect concentrations (EC_{20}, EC_{IR2}) up to the highest analyzed concentration (HAC) measured for both, migrates and extracts, were plotted. Relative estrogenic (*C*) and antiandrogenic (*D*) activities were correlated at 3.75 mg plastic $well^{-1}$. Sample numbers are given if migrate and extract shared a similar toxicity (between dotted lines) or if the migrate was more toxic (above upper dotted line). Note: LOD, limit of detection.

Table 2. Chemical features detected in the plastic extracts and migrates and tentatively identified compounds.

	Number of features						Tentatively identified chemicals			
	Total	Extract only	Low migration	Readily leachable	Migrate only	Sum in migrate[b,c]	CPPdb	ECHAdb	NORMAN	Combined (%)[c]
HDPE 1	1790	740	1582	146	62	208 (12)	2	4	18	19 (1.1)
LDPE 1[a]	1129	383	845	199	85	284 (25)	2	5	22	23 (2.0)
LDPE 2[a]	6297	2106	5797	351	149	500 (8)	15	18	37	37 (0.6)
LDPE 3[a]	8359	2300	5940	1430	989	2419 (29)	59	76	154	155 (1.9)
LDPE 4	8415	1169	3315	4476	624	5100 (61)	379	443	798	810 (9.6)
PS 1[a]	1851	334	768	537	546	1083 (59)	28	34	108	112 (6.1)
PS 2[a]	6547	1626	3949	2054	544	2598 (40)	58	90	266	268 (4.1)
PS 3[a]	685	296	613	64	8	72 (11)	0	0	1	1 (0.1)
PS 4	896	414	879	17	0	17 (2)	0	0	1	1 (0.1)
PP 1[a]	7322	1148	4077	2772	473	3245 (44)	211	284	590	597 (8.2)
PP 2[a]	16,122	4294	10,766	3744	1612	5356 (33)	263	351	670	679 (4.2)
PET 1[a]	2159	986	2101	49	9	58 (3)	1	1	2	2 (0.1)
PVC 1[a]	11,884	2057	7730	3985	169	4154 (35)	312	402	817	822 (6.9)
PVC 2	6630	1368	4329	1692	609	2301 (35)	49	76	193	198 (3.0)
PVC 3	5621	1585	4889	646	86	732 (13)	34	41	85	86 (1.5)
PVC 4	11,986	2474	8241	3457	288	3745 (31)	248	297	585	588 (4.9)
PUR 1	12,617	2512	5907	5865	845	6710 (53)	56	63	146	149 (1.2)
PUR 2	9402	759	1527	7574	301	7875 (84)	53	63	120	121 (1.3)
PUR 3	17,973	3873	9037	8572	364	8936 (50)	116	158	314	317 (1.8)
PUR 4	3759	1112	2848	652	259	911 (24)	22	35	90	92 (2.4)
PLA 1[a]	5071	2077	4846	212	13	225 (4)	2	2	19	20 (0.4)
PLA 2[a]	4956	2256	4877	74	5	79 (2)	0	0	2	2 (0.0)
PLA 3	12,615	2962	6430	5125	1060	6185 (49)	189	260	534	544 (4.3)
PLA 4[a]	6425	2314	6337	81	7	88 (1)	4	6	15	15 (0.2)

Note: [a] food contact materials, [b] sum of readily leachable and migrate only, [c] % of total.

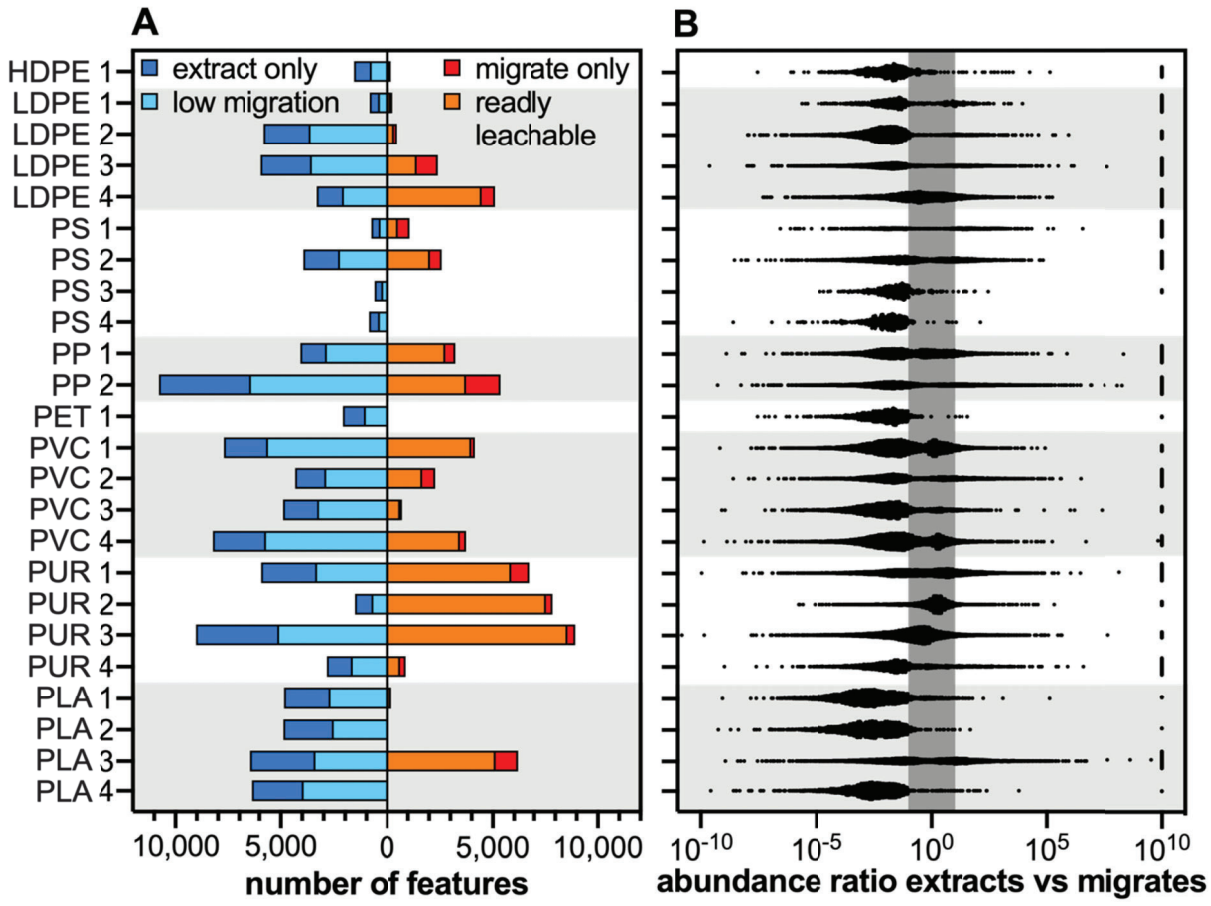

Figure 3. Numbers of chemical features migrating from plastic products (*A*) and ratios of the abundance of each feature in the extract and the migrate (*B*). The left side of each graph represents features with no/low migration, meaning those only present in extracts or with a migration ratio of < 0.1, the right side represents features that are readily leachable, that is, they are only detected in migrates (in *B*: ratio of 1×10^{10}) or migrating with a ratio of > 0.1. The dark grey band in *B* highlights the area in which abundance of features is similar (maximally 10-fold lower or higher) in the extracts and migrates.

A4 What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*

What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*

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Declaration of author contributions to the publication:

	LZ	SG	JO	MW	CV
Concept and design	**70%**	–	5%	5%	20%
Conducting tests and experiments	**30%**	70%	–	–	–
Compilation of data sets and figures	**80%**	20%	–	–	–
Analysis and interpretation of data	**85%**	10%	–	–	5%
Drafting of manuscript	**79%**	1%	5%	5%	10%

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What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*☆

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ABSTRACT

Given the ubiquitous presence of microplastics in aquatic environments, an evaluation of their toxicity is essential. Microplastics are a heterogeneous set of materials that differ not only in particle properties, like size and shape, but also in chemical composition, including polymers, additives and side products. Thus far, it remains unknown whether the plastic chemicals or the particle itself are the driving factor for microplastic toxicity. To address this question, we exposed *Daphnia magna* for 21 days to irregular polyvinyl chloride (PVC), polyurethane (PUR) and polylactic acid (PLA) microplastics as well as to natural kaolin particles in high concentrations (10, 50, 100, 500 mg/L, $\leq$ 59 μm) and different exposure scenarios, including microplastics and microplastics without extractable chemicals as well as the extracted and migrating chemicals alone. All three microplastic types negatively affected the life-history of *D. magna*. However, this toxicity depended on the endpoint and the material. While PVC had the largest effect on reproduction, PLA reduced survival most effectively. The latter indicates that bio-based and biodegradable plastics can be as toxic as their conventional counterparts. The natural particle kaolin was less toxic than microplastics when comparing numerical concentrations. Importantly, the contribution of plastic chemicals to the toxicity was also plastic type-specific. While we can attribute effects of PVC to the chemicals used in the material, effects of PUR and PLA plastics were induced by the mere particle. Our study demonstrates that plastic chemicals can drive microplastic toxicity. This highlights the importance of considering the individual chemical composition of plastics when assessing their environmental risks. Our results suggest that less studied polymer types, like PVC and PUR, as well as bioplastics are of particular toxicological relevance and should get a higher priority in ecotoxicological studies.

1. Introduction

Microplastics are ubiquitous in natural environments and experimental studies have shown that they can induce a wide range of negative impacts in marine and freshwater species across the animal kingdom (Sá et al., 2018; Triebskorn et al., 2019). However, the evaluation of toxicity is complicated by the fact that microplastics are not one homogenous entity (Lambert et al., 2017). They originate from many different product types, are composed of various polymers, chemical additives and side products and differ in particle properties (Rochman et al., 2019). Up to date, few studies have addressed this heterogeneity of materials from a comparative perspective. As an example, the effects of mostly spherical microplastics are investigated. In contrast, irregular fragments and fibers originating from abrasion and fragmentation of plastic products (secondary microplastics) are predominant in the environment but less frequently considered (Burns and Boxall, 2018). At the same time, irregular microplastics might be more toxic than their spherical counterparts, for instance in terms of acute (Frydkjær et al., 2017) and chronic effects in daphnids (Ogonowski et al., 2016). In addition, research focuses only on few polymer types, most often on polystyrene (PS) and polyethylene (PE) particles, disregarding other polymer types of high production and consumption, such as polypropylene (PP) and polyvinyl chloride (PVC; PlasticsEurope, 2015; Sá et al., 2018). However, the toxicity of

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microplastics may also depend on the polymer type or on the chemicals that a plastic product, and therefore its fragments, contain (Renzi et al., 2019). One single plastic product can contain hundreds of chemicals (Zimmermann et al., 2019). These include additives like antioxidants, flame retardants, plasticizers and colorants as well as residual monomers and oligomers, side-products of polymerization and compounding and impurities (Muncke, 2009). Most of them are bound to the polymer matrix only via weak van der Waals forces and, therefore, can leach into the surrounding environment and become available for aquatic organisms (Andrady, 2011; Oehlmann et al., 2009). Once taken up, these plastic chemicals can entail negative impacts. For instance, aqueous leachates from epoxy resin or PVC plastic products induced acute toxicity in *Daphnia magna* (Lithner et al., 2012). Still, studies on the contribution of plastic chemicals to microplastic toxicity are scarce.

Thus, our study aims to elucidate whether the chemicals present in plastics contribute to microplastic toxicity in the well-studied model organism *D. magna*. We produced irregular microplastics from three polymer types that are less frequently studied, including polyurethane (PUR) and polyvinyl chloride (PVC) that often contain high amounts of chemicals (Zimmermann et al., 2019) as well as the bio-based, biodegradable polylactic acid (PLA). We also included kaolin particles as a reference to evaluate whether microplastics are more toxic than natural particles. Since our aim was to compare the contribution of plastic chemicals and particles to the toxicity, we used high concentrations that are not environmentally relevant but induced adverse effects. First, we compared the effects of all microplastic types on mortality, reproductive output, timing of reproduction and body lengths of *D. magna* in a chronic exposure experiment. In a second experiment, we evaluated whether plastics chemicals contribute to microplastic toxicity. For this, we studied the effects of untreated microplastics and microplastics from which we removed the extractable chemicals as well as the extractable chemicals (worst-case scenario) and the chemicals migrating into water (realistic scenario), alone.

2. Materials & methods

2.1. Test materials

We purchased a floor covering, a scouring pad and a shampoo bottle in local retailer stores to produce irregular microplastics. The products are made of petroleum-based PVC and PUR as well as the bio-based and biodegradable PLA. These materials were selected based on our previous results in the Microtox assay (Zimmermann et al., 2019). In the assay the inhibition of bioluminescence of the bacterium *A. fischeri* indicates baseline toxicity. Since the latter generally correlates well with toxicity in *D. magna* (Kaiser, 1998), we chose products that induced a high baseline toxicity in the Microtox assay (Zimmermann et al., 2019, PVC corresponds to PVC 4, PUR to PUR 1, PLA to PLA 3). In our previous study, we confirmed the polymer types using Fourier-transform infrared spectroscopy and characterized the chemicals present in the products by performing non-target, high-resolution gas chromatography–mass spectrometry.

2.2. Production of microplastics

Whenever feasible, we used glass consumables to avoid sample contamination, rinsed all materials twice with acetone (pico-grade, LGC Standards) and annealed glass items at 200 °C for ≥3 h. The content was removed from packaging samples and the products were rinsed thoroughly with ultrapure water until all residues were removed. Plastic items were cut into small pieces (~0.5 cm^2), frozen in liquid nitrogen and ground in a ball mill (Retsch MM400, Retsch GmbH, Germany) at 30 Hz for 1 min. The process of freezing and grinding was repeated 6–10 times to produce sufficient amounts of plastic powder. The plastic powder and kaolin (~$Al_2Si_2O_5(OH)_4$, CAS 1332-58-7, Merck, Darmstadt, Germany) were sieved to ≤59 μm for particle characterization and the experiments. To this end, polyester mesh (RCT Reichelt Chemie Technik GmbH + Co, Heidelberg, Germany) with respective mesh sizes were fixed horizontally in a custom-made sieving device that was mounted on a sediment shaker (Retsch AS 200 basic, Retsch GmbH, Germany) and was shaken at 80–100 Hz for 10 min. With a size of ≤59 μm all particles are in a size range which can be ingested by *D. magna* (Burns, 1968).

2.3. Preparation and characterization of stock suspensions

We prepared microplastic stocks by suspending between 0.2 and 500 mg of particles/L Elendt M4 medium (Elendt and Bias, 1990) and shaking it at 80 rpm for ≥24 h (GFL-Kreis-Schüttler 3017, Gesellschaft für Labortechnik GmbH, Burgwedel, Germany). We used mass-based concentrations, because we aimed at comparing the toxicity of the chemicals present in the different plastics based on the same mass, not particle number. The corresponding numerical particle concentrations and size distributions were also determined using a Coulter counter (Multisizer 3, Beckman Coulter, Germany; orifice tube with 100 and/or 400 aperture diameter for a particle size range of 2.0–60 μm and 8.0–240 μm, respectively). For this, 1.0–2.5 mL of the particle suspension were taken from the middle of the exposure vessel or flask (continuously stirred) and transferred immediately to the Coulter counter medium (100 mL sterile-filtrated 0.98% sodium chloride, continuously stirred). In addition to the samples, we also analyzed the pure sodium chloride as a blank and the Elendt M4 medium as microplastic-free control medium. The kaolin particles were treated identically like the microplastics. All samples were analyzed in three to ten replicates. The blank corresponding to each measurement was analyzed in triplicates.

2.4. Microplastic characterization

For an initial characterization and comparison of our microplastics regarding size distribution, shape, surface morphology and behavior in suspension, we prepared suspensions with 0.2, 2.0, 20.0, 60.0 (not measured for PLA and kaolin), 100 and 500 mg microplastics or kaolin/L Elendt M4 medium. We determined particle size distributions (Fig. S1) as well as numerical particle concentrations using a Coulter counter (see 2.3.). From the latter, we obtained calibration curves by linear regression for mass (mg) vs. numerical particle concentration/L for each plastic type. We corrected the latter for the mean particle concentration in the respective control measurement (microplastic-free Elendt M4 medium; Fig. S2). In order to assess particle shape and surface morphology, we took images with a Hitachi S-4500 scanning electron microscope (SEM; Fig. 1). Additionally, stock suspension containing 500 mg microplastics or kaolin/L were visually examined for the distribution of particles in the water column and for agglomeration immediately after shaking and after resting for two and seven days.

2.5. Culture of test organism Daphnia magna

D. magna were obtained from IBACON GmbH (Rossdorf, Germany). Ten individuals were cultured in 1 L of Elendt M4 medium (Elendt and Bias, 1990) at a constant temperature of 20 ± 1 °C and a photo-period of 16:8 h light:dark for approximately 28 days. Juveniles were removed thrice a week and daphnids were fed with a suspension of live green algae (*Desmodesmus subspicatus*), cultured

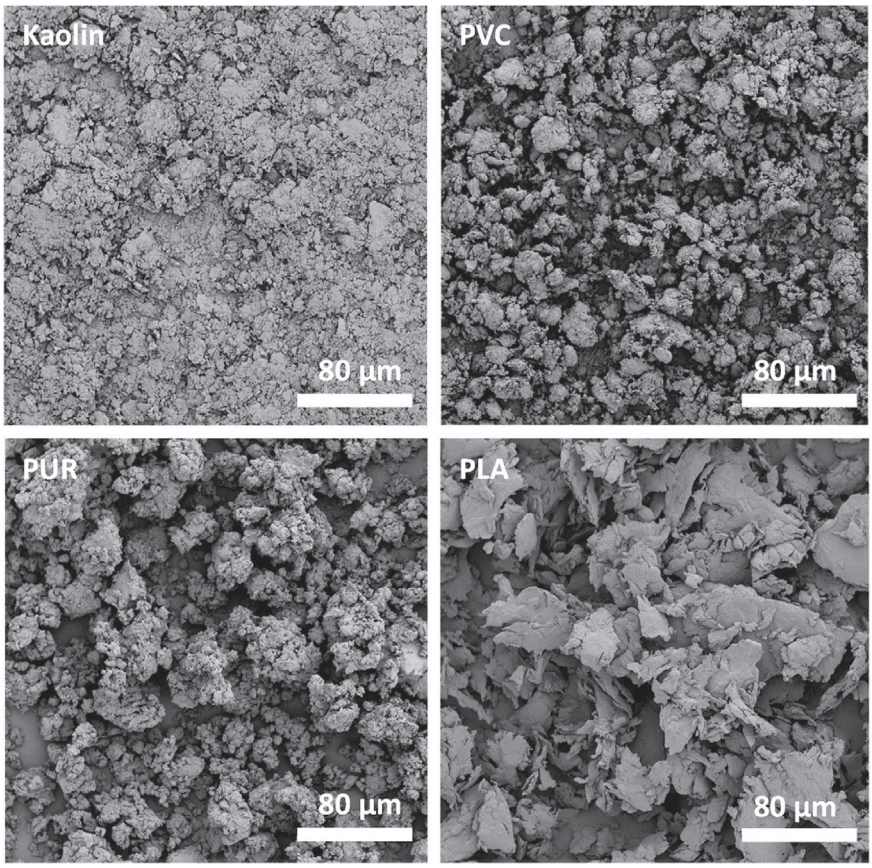

Fig. 1. Scanning electron microscope (SEM) images of kaolin as well as PVC, PUR and PLA microplastics (300 × magnification).

according to OECD guideline (OECD, 2012) supplying 0.15 mg carbon per individual per day. Once a week, the medium was completely renewed.

2.6. Chronic toxicity of microplastics on Daphnia magna

Prior to toxicity experiments, we evaluated qualitatively whether *D. magna* ingests PVC, PUR and PLA microplastics. PVC and PLA particles were stained with Nile red (CAS 7385-67-3, reinst; Carl Roth GmbH + Co. KG, Karlsruhe, Germany) for visualization adapting the method of Erni-Cassola et al. (2017). Six starved individuals which were 6 d old were exposed to a 250 mg/L microplastic suspension at culturing conditions. After 24 h, we analyzed ingestion using an Olympus BX50 fluorescence microscope (Olympus Europa SE & Co. KG, Hamburg, Germany).

To analyze and compare the effects of microplastics and kaolin particles, we conducted chronic exposure experiments with *D. magna* according to OECD guideline 211 (OECD, 2012). In brief, neonates (<24 h old) of the third or fourth brood were exposed individually for 21 d in 100 mL glass beakers containing 50 mL Elendt M4 medium. Microplastic suspensions were prepared as stocks and continuously stirred during the transfer to the test vessels. After dilution with Elendt M4 medium to the desired exposure concentrations of 10, 50, 100 and 500 mg/L, we determined the size distributions (see 2.3) and the numerical particle concentrations corrected for the mean particle concentration in the control (Elendt M4 medium, Table S1). We selected such high concentrations because they induced adverse effects in *D. magna* in previous experiments conducted in our laboratory (unpublished data). We used 10 replicates per treatment and 20 negative controls (NC) in each experiment. Experiments were conducted at a 16:8 h light:dark cycle at 20 ± 1 °C and beakers were covered with watch glasses to reduce evaporation. Animals were fed daily with *D. subspicatus* according to OECD guideline 211 (OECD, 2012) and the test medium was completely renewed thrice a week by transferring the daphnids into new vessels. Each day, we recorded the mortality of adult daphnids (15 s immobility after agitation; OECD, 2004) and their reproductive output (number of neonates per female). We also recorded the day of first brood (timing of reproduction) and the total number of live offspring for each surviving parent organisms throughout the experiment. Surviving adults were preserved in 70% ethanol. Their size was determined using a stereo microscope (Olympus SZ61, Olympus GmbH, Germany) and the software Diskus (version 4.50.1458) by measuring the distance between the center of the eye and the base of the apical spinus (Ogonowski et al., 2016). We observed that eight out of 180 individuals, randomly distributed across all treatments, had >40% lower body length compared to the other animals and did not reproduce. We sexed these animals according to Mitchell (2001) and identified them as females. Microplastic concentrations reducing the reproduction by 50% compared to the negative control ($EC_{50Repro}$) were used in the second experiment (2.7.). We excluded the smaller individuals mentioned above from the calculation of the $EC_{50Repro}$ because we could not estimate an EC_{50} when they were included.

2.7. Contribution of plastic chemicals to microplastic toxicity

In order to analyze whether the chemicals present in and leaching from plastics induce the observed effects, we conducted a second chronic exposure experiment with *D. magna*. Generally, the setup and endpoints were identical as before (2.6.) but in this second experiment daphnids were exposed to four treatments reflecting four exposure scenarios (Fig. 2):

(1) PVC, PUR and PLA microplastics containing all chemicals (MP).
(2) PVC, PUR and PLA microplastics extracted with methanol. Thus, they do not contain extractable chemicals (eMP).
(3) The corresponding plastic extracts (E) containing all chemicals that can be extracted with methanol. The extracts represent a worst-case scenario because extraction with an organic solvent will release most of the chemicals present in the material.
(4) Plastic migrates (M) containing the chemicals released from PVC, PUR and PLA microplastics into the water, thus, representing a more realistic scenario.

For preparing the suspensions (MP, eMP) and leachates (E, M) of each microplastic type, we used the respective mass concentrations that reduced reproduction by half in the first experiment ($EC_{50Repro}$, PVC: 45.5 mg/L, PUR: 236 mg/L, PLA: 122 mg/L). This means that for each microplastic type, suspensions for scenario 1 and 2 were prepared using the same mass concentrations. Scenarios 3 and 4 contained the chemicals extracted or migrating from the very same mass to ensure comparability. Specifically, the suspensions and leachates for the four exposure scenarios were prepared as follows:

(1) MP stock suspensions were prepared as described in 2.3.

(2 + 3) Extracted microplastics and the extracts were produced by weighing microplastics in amber glass vials and adding 13.5 mL methanol (99.9% LC-grade, Sigma-Aldrich, exception PUR: 16.5 mL). We selected methanol as solvent because it does not dissolve the polymers. After sonication in an ultrasound bath for 1 h at room temperature, the suspensions were vacuum-filtrated over a polyethersulfone membrane (pore size: 1 μm, Sartorius Biolab Products, Satorius Stedim Biotech GmbH, Goettingen, Germany) precalibrated with methanol to separate the extract from the extracted particles. The extracted particles were dried at 30 °C for 24 h, the dry weight was recorded and eMP stock suspensions were prepared as described in 2.3. The extracts were transferred into clean glass vials and dimethyl sulfoxide (DMSO, Uvasol, Merck) was added as a keeper. The volume of DMSO was dependent on the recovered extract volume to adjust to the plastic concentrations corresponding to the $EC_{50Repro}$ used in scenarios 1 and 2. Extracts were evaporated under a gentle stream of nitrogen and stored at −20 °C prior to use. Exposure vessels were spiked with 5 μl extract.

(4) Migrates were prepared by suspending microplastic masses corresponding to the $EC_{50Repro}$ used in scenarios 1 and 2 in 5.5 L Elendt M4 medium 48 h before the start of the experiment. Directly prior to the initial set up of the experiment as well as each medium renewal, 500 mL of that migrate suspensions were filtrated over a polyethersulfone membrane with a pore size of 1 μm to remove the particles and 50 mL aqueous migrate were transferred into each test vessel. In that way, the migration of chemicals proceeded in parallel to the experiment.

In order to exclude effects of the solvent or a potential contamination, we included a solvent control (DMSO only) and procedural blanks of the extraction (PB E) and the migration (PB M) consisting of Elendt M4 media treated identically as the plastic extracts and migrates, respectively.

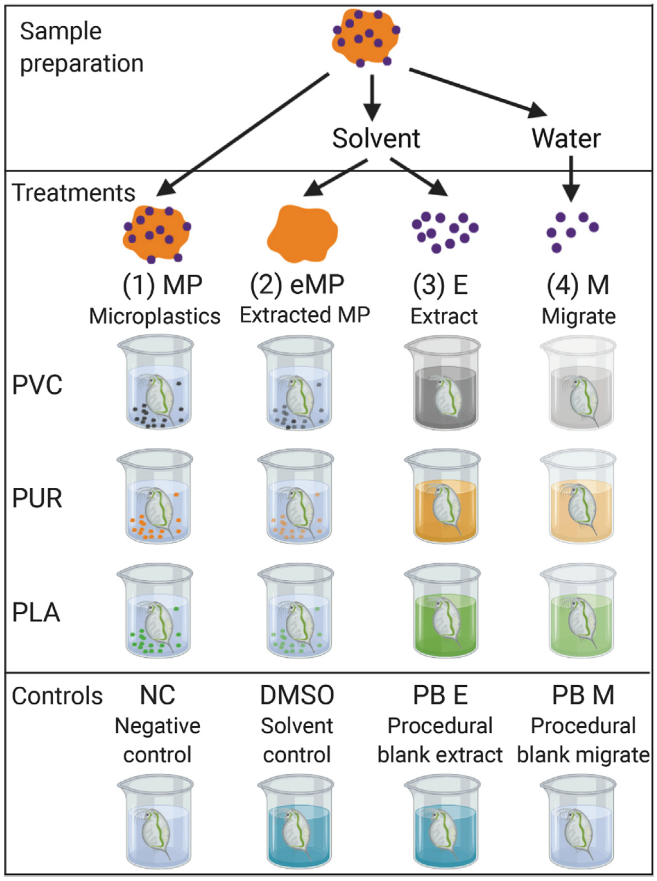

Fig. 2. Setup of the second experiment. Daphnids were exposed to four treatments of PVC, PUR and PLA: (1, MP) untreated microplastics containing all chemicals, (2, eMP) microplastics without extractable chemicals, (3, E) plastic extracts containing all extractable chemicals and (4, M) plastic migrates containing the chemicals released from microplastics into water (M). We included a negative control (NC), a solvent control (DMSO) and procedural blanks of the extraction (PB E) and migration (PB M) consisting of Elendt M4 media treated identically as the plastic extracts and migrates, respectively.

2.8. Data analysis

We used GraphPad Prism 5 (GraphPad Software, San Diego, CA) for regressions and statistical analyses. Continuous life-history data were checked for normal distribution (D'Agostino-Pearson tests for $n \geq 8$ or Kolmogorov-Smirnov tests for $n = 5–7$). Since all data was not normally distributed, we used non-parametric Kruskal-Wallis with Dunn's multiple comparison post-test to assess differences between treatments and negative controls. Fisher's exact test was applied for categorical data. The significance level was set at $p < 0.05$. The 10% and 50% effect concentrations (EC_{10} and EC_{50}) for reproduction were determined using a four-parameter logistic model and were compared using the extra sum-of-squares F test. We indicate the F value together with the degrees of freedom numerator (DFn) and denominator (DFd). Since solvent control (DMSO), extraction (PB E) and migration (PB M) procedural blanks did not differ significantly from the negative control, we pooled all controls (C).

3. Results

3.1. Characterization of microplastics

To characterize the microplastics and kaolin used in our study, we compared the numerical particle concentrations at identical mass concentrations, the size distributions, shapes and surface morphology as well as behavior in suspension prior to experiments. For the highest mass-based concentration (500 mg/L), the numerical concentrations were 8.38×10^7 particles/L (PUR), 1.35×10^8 particles/L (PVC) and 2.08×10^8 particles/L (PLA, Fig. S2). Thus, the PLA suspension contained 1.6 times more particles than the PVC suspension and 2.5 times more than the PUR suspension. While at 100 mg/L, the numerical concentrations of all microplastics were very similar and only differed by a maximum factor of 1.2, the differences increased again towards lower mass concentrations. Correspondingly, at the lowest mass-based concentration (0.2 mg/L), numerical concentration were 3.77×10^6 particles/L (PLA), 1.35×10^7 particles/L (PUR) and 1.63×10^7 particles/L (PVC). That 100 mg/L concentrations were most similar to each other while differences between microplastic types increased towards lower and higher concentrations was also true for the concentrations in the exposure vessels. Here, the numerical concentrations varied by a maximum factor of 4.0 for 10 mg/L, of 1.8 for 100 mg/L and 2.2 for 500 mg/L between the three polymers (Table S1). In contrast, kaolin suspensions contained 11–50 times more particles at same mass concentrations.

The size distributions of all microplastics of our study are very similar (Fig. S1). Independent of the particle type, the number of particles increases with decreasing sizes. Whereas the majority of kaolin particles is <10 μm, microplastics contain higher relative particle quantities at sizes up to about 20 (PVC) or 40 μm (PUR, PLA). All particles have irregular shapes and rough surfaces (Fig. 1). While PVC, PUR and kaolin particles are rather round, PLA particles are flatter and disc-shaped. After preparation of stocks, including ≥24 h of shaking, all microplastic types and kaolin were homogenously distributed in the water column. Kaolin remained suspended in the water phase after two and seven days without moving the suspensions, whereas most microplastics sedimented and few floated on the surface. Although daphnids are primarily filter feeders, they also graze on sediments and we observed them at the bottom of the test vessels. Thus, all microplastic types are available to the daphnids. A qualitative uptake experiment demonstrated that PVC, PUR and PLA microplastics are readily ingested by *D. magna* since they were visible in the gastrointestinal tract (Fig. S3).

3.2. Chronic effects of microplastics on Daphnia magna

To investigate whether microplastics affect life-history traits of *D. magna* and whether toxicity changes with the plastic type, we exposed daphnids to PVC, PUR, PLA and kaolin particles. All microplastics reduced the reproductive output of *D. magna* (Fig. 3A) with an efficiency and effect level specific to the plastic type. PVC impaired the reproduction the most with an EC_{50} of 45.5 mg/L (Table 1) and significantly decreased the number of neonates from 101 per adult (control) to 34 at 100 mg/L and to 0 at 500 mg/L (Fig. 3A). Exposure to PLA and PUR microplastics reduced the reproduction significantly compared to the control at 500 mg/L with EC_{50} values of 122 and 236 mg/L, respectively. While an exposure to 10 and 50 mg/L of kaolin increased the reproduction to 130 and 110 neonates/animal ($p > 0.05$), 500 mg/L significantly reduced the mean number of neonates per surviving female (21 neonates/animal) to values similar to PLA. With an EC_{50} of 275 mg/L, kaolin was less efficient than microplastics in affecting reproduction. In addition, exposure to 500 mg/L PVC and kaolin significantly delayed the reproduction and the mean day of the first brood occurred eight and four days later than in the control animals, respectively (Fig. S4).

Using the same data, we also compared the reproductive output based on numerical particle concentrations (Fig. 3B). With an EC_{50} of 1.14×10^7 particles/L, PVC was most efficient in reducing the reproduction, followed by PLA (EC_{50} of 5.13×10^7 particles/L) and PUR (EC_{50} of 7.29×10^7 particles/L, Table 1). With an EC_{50} of 2.61×10^9 particles/L, kaolin was >35 times less toxic than all three microplastics. A statistical comparison of the EC_{50} values of the four particle types demonstrated that all values, both, if based on masses ($F = 9.09$ ($DFn = 3$, $DFd = 119$)) or numerical particle concentration ($F = 61.76$ ($DFn = 4$, $DFd = 135$)), differed significantly from each other ($p < 0.05$).

Except for PLA, the impacts of the particle exposure on daphnid survival were low with 10 mg/L PVC and 50 mg/L kaolin inducing a maximum of 30% mortality (Fig. S5). An exposure to PLA increased the mortality in a concentration-dependent manner to 60% at 500 mg/L. The mortality in the controls was 5%.

The mean body length of adult *D. magna* was significantly lower in animals exposed to 500 mg/L of microplastics (Fig. S6). Control animals were 4.10 mm in size compared to 3.48, 3.57 and 3.30 mm in specimens exposed to PVC, PUR and PLA, respectively. Exposure to the 500 mg kaolin/L also reduced the size of daphnids similar to PLA.

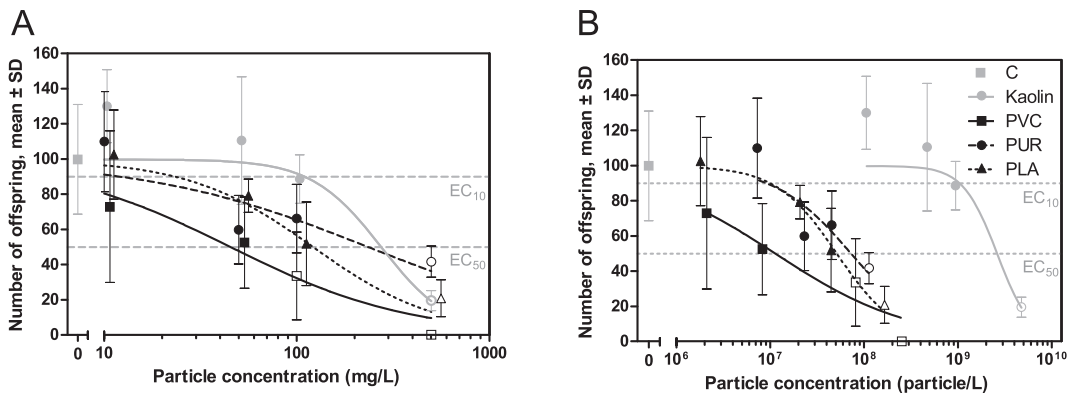

Fig. 3. Effects of a chronic exposure of *Daphnia magna* to kaolin, PVC, PUR and PLA particles on the reproduction. Data is presented as mass-based (A) and numerical concentrations (B). The latter were corrected for mean particle concentration in the blank (M4 medium). Open symbols indicate significant differences ($p < 0.01$) compared to control animals (C). EC_{10}, EC_{50}: concentrations inducing 10 and 50% effect, SD: standard deviation.

Table 1
Mass-based and numerical particle concentrations of kaolin as well as PVC, PUR and PLA microplastics reducing the reproduction of *Daphnia magna* by 10% (EC_{10}) and 50% (EC_{50}).

Treatment	EC_{10} (mg/L)	EC_{50} (mg/L)	EC_{10} (particle/L)	EC_{50} (particle/L)
Kaolin	111 (43.8–279)	275 (174–435)	1.05×10^9 (4.12×10^8–2.66×10^9)	2.61×10^9 (1.64×10^9–4.14×10^9)
PVC	n.a.[a]	45.5 (26.8–77.2)	n.a.[a]	1.14×10^7 (6.96×10^6–2.61×10^7)
PUR	12.4 (3.01–50.7)	236 (120–463)	9.26×10^6 (3.26×10^6–2.63×10^7)	7.29×10^7 (4.58×10^7–1.16×10^8)
PLA	23.6 (9.62–58.0)	122 (79.9–186)	1.07×10^7 (3.97×10^6–2.56×10^7)	5.13×10^7 (3.50×10^7–7.50×10^7)

[a] EC_{10} below the lowest measured concentration of 10 mg/L; The 95% confidence intervals are given in brackets.

3.3. Contribution of plastic chemicals to microplastic toxicity

Next, we evaluated whether the observed toxicities of microplastics are caused by plastic chemicals. For this, we exposed *D. magna* to microplastics containing all chemicals (MP), extracted microplastic particles (eMP), the chemicals extracted from PVC, PUR and PLA microplastics (E) and the chemicals migrating from the microplastics to aqueous medium (M, Fig. 2). In order to ensure comparability, the exposure concentrations were based on the $EC_{50Repro}$ that we derived from the first experiment (PVC: 45.5 mg/L; PUR: 236 mg/L, PLA: 122 mg/L).

For PVC, exposure to the extracted chemicals (E) but not the plastic particles (MP and eMP) reduced significantly the reproductive output from 117 (control) to 25 neonates/animal (Fig. 4A). Along that line, exposure to the PVC extract (E) also delayed the reproduction by three days (Fig. 4D) and reduced the body lengths of daphnids (4.08 vs. 4.56 mm in control animals, Fig. S7A). The chemicals migrating to aqueous medium (M) did not have a significant effect.

In comparison, the toxicity of PUR and PLA microplastics in *D. magna* was mediated by the particle properties and not the chemicals. Here, the microplastics and extracted microplastics significantly reduced the reproduction (Fig. 4B and C) as well as the size of daphnids (Figs. S7B and C). Extracted PUR particles also delayed the day of the first brood by 1–3 days compared to the control (Fig. 4E). In line with the first experiment, PLA was the only microplastic type inducing mortality. This effect was mediated by the particles and not the chemicals (Fig. 5).

To further evaluate if and which particle characteristics might be responsible for the deviating toxicities, we analyzed differences in numerical concentrations (particle count), size distribution as well as the shape and surface morphology of original and extracted microplastics. Regarding particle numbers, the suspension of extracted PVC particles contained 1.89×10^8 particles/L compared to 0.50×10^8 particles/L in the suspension of the PVC microplastics (Fig. 6). Although both suspensions were prepared using the same mass, the extracted microplastic suspension had a 3.8 times higher particle concentration. Comparisons of the particle size

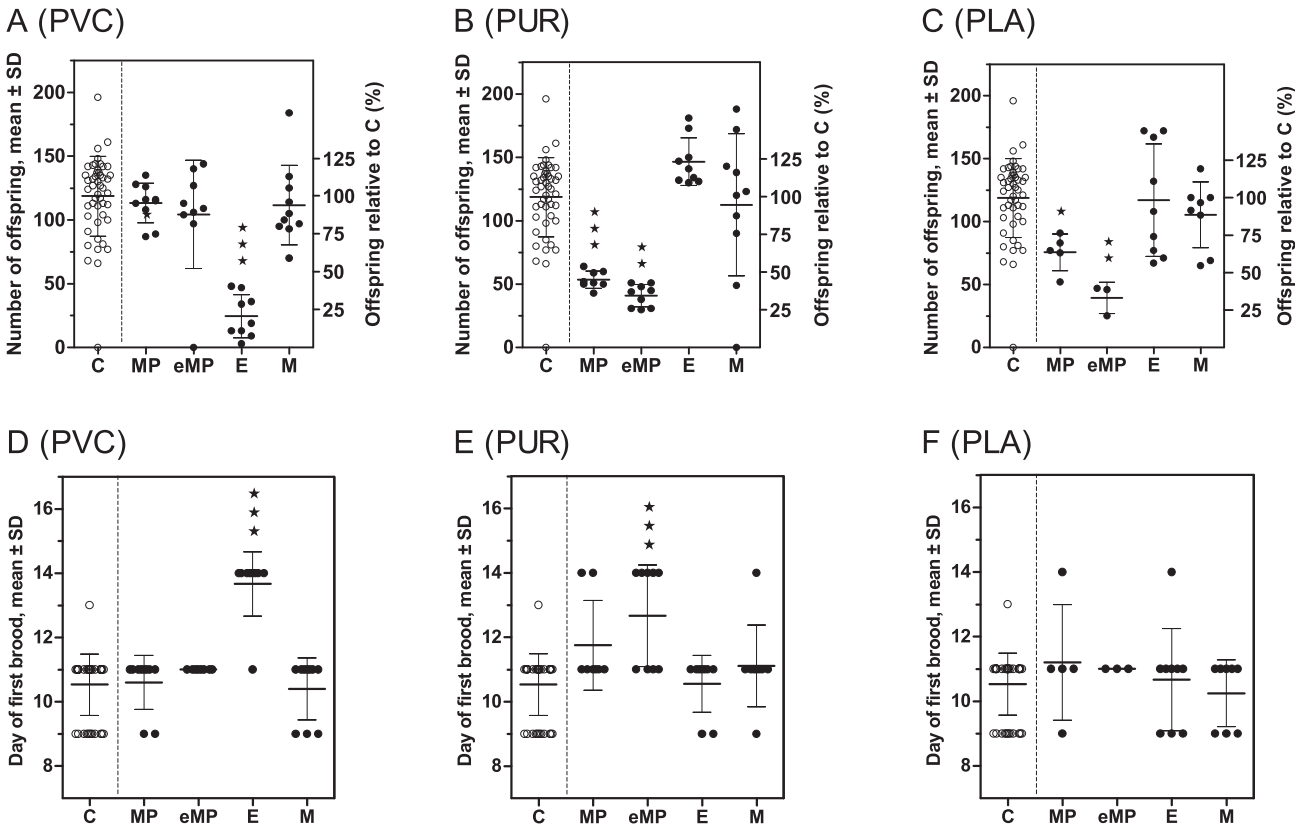

Fig. 4. Effect of a chronic exposure of *Daphnia magna* to PVC (45.5 mg/L), PUR (236 mg/L) and PLA (122 mg/L) microplastics on the reproductive output (A–C) and the timing of reproduction (D–F). Treatments include microplastics (MP), microplastics without extractable chemicals (eMP), the chemicals extracted (E) and migrating from microplastics to aqueous medium (M). Asterisks indicate significant differences to the controls (C) with ★ $p < 0.05$, ★★ $p < 0.01$, ★★★ $p < 0.001$ (Kruskal-Wallis with Dunn's multiple comparison post-test).

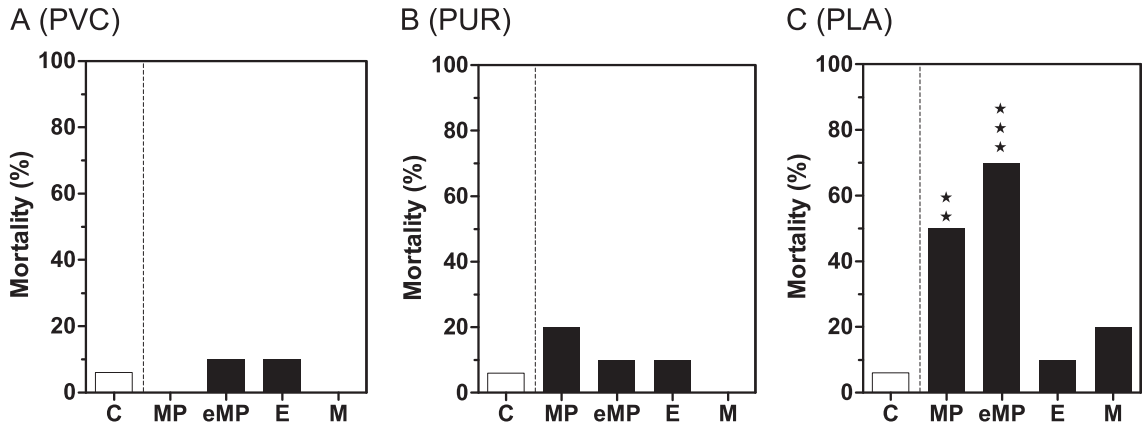

Fig. 5. Mortality of *Daphnia magna* after 21 days exposure to 45.5 mg/L PVC (A), 236 mg/L PUR (B) and 122 mg/L PLA (C) microplastics. Treatments include microplastics (MP), microplastics without extractable chemicals (eMP), the chemicals extracted (E) and migrating from microplastics to aqueous medium (M). Asterisks indicate significant differences to the controls (C): ★★ $p < 0.01$, ★★★ $p < 0.001$ (Fisher's exact test, comparison to C).

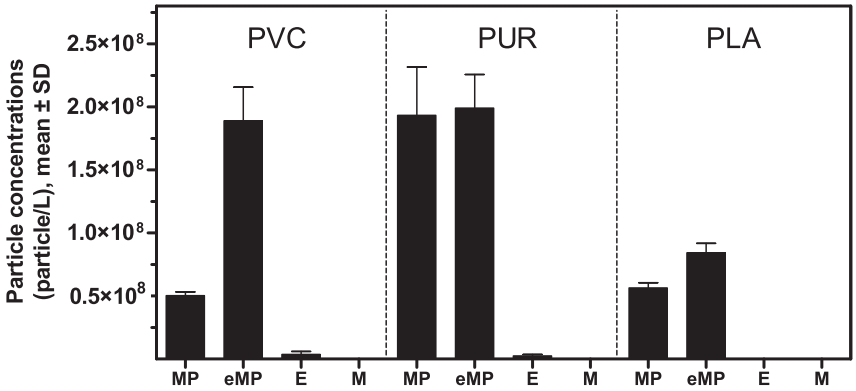

Fig. 6. Numerical particle concentrations in the treatment suspension of the second experiment. Treatments include microplastics (MP), microplastics without extractable chemicals (eMP), the chemicals extracted (E) and migrating from microplastics to aqueous medium (M). SD: standard deviation.

distributions showed that the extracted PVC particles are smaller than the untreated ones (Fig. S8). Suspensions of the original and extracted PUR microplastics contained approximately the same particle concentration (1.93×10^8 and 1.99×10^8 particles/L) like extracted PVC. The numerical concentrations of microplastics in the PLA suspensions were approximately 2.8 times lower (MP: 0.57×10^8 particles/L, eMP: 0.84×10^8 particles/L; Fig. 6). The extraction of PUR and PLA microplastics did not change their size distribution (Fig. S8). SEM imaging demonstrated that the extraction did not alter shapes nor surface morphologies of any of the microplastic types (Fig. S9).

4. Discussion

4.1. Microplastic effects on Daphnia magna depend on the plastic type

For the hazard assessment of microplastics, it is crucial to consider the diverse picture of synthetic polymers entering the environment (Lambert et al., 2017; Rochman et al., 2019). However, the physical and chemical heterogeneity of microplastics has rarely been reflected in ecotoxicological studies to date. To address this knowledge gap, we compared the impact of so far understudied PVC, PUR and PLA particles on *D. magna* upon chronic exposure. Since we aimed at understanding the chemical and physical toxicity of microplastics and not their environmental risks specifically, we used high concentrations that caused negative impacts in *D. magna* but are clearly much higher than currently occurring in freshwater ecosystems.

In this range, all three microplastics affected life-history traits of *D. magna*. While PVC microplastics were most potent in decreasing (at 10–500 mg/L) and delaying reproduction (at 500 mg/L), PLA was in reducing survival (at 500 mg/L). When comparing reproductive outputs based on numerical concentrations, we observed a similar picture, with PVC being more potent in decreasing reproduction ($EC_{50} = 1.14 \times 10^7$ particle/L) than PLA (5.13×10^7 particle/L) and PUR (7.29×10^7 particle/L). Thus, impacts of microplastics depend on the polymer type and the endpoint under investigation.

Besides our toxicity study, only few others have analyzed polymers other than PS and PE or compared different microplastic types. Two studies compared PE and polyethylene terephthalate (PET) microplastics from consumer plastics and observed neither acute effects at mass concentrations comparable to our study (particle size: 23–264 μm; concentration: 100 mg/L; Kokalj et al., 2018) nor chronic impacts (exposure concentration based on surface area; Trotter et al., 2019) on daphnids. So far, toxicity data for PUR particles are unavailable but some data for PVC and PLA microplastics exists. Irregular PLA microplastics (3.4 μm; 19.6 μg/L) did not affect feeding, size and population growth of *D. magna* upon chronic exposure (Gerdes, 2018). In a comparative analysis of irregular PVC, PP and PE particles (10–100 μm; 50 mg/L), PP and PE induced a higher acute toxicity than PVC on *D. magna* under fasting

conditions (Renzi et al., 2019). Schrank et al. (2019) compared irregular rigid and flexible PVC (4–276 μm) and reported delay of primiparity in *D. magna* upon exposure to rigid PVC and alterations in body lengths and reproductive output for flexible PVC. This indicates that the toxicity of microplastics not only depends on the polymers type but also differs between plastics made of the same polymer.

Comparison of microplastics to the natural kaolin particle demonstrates that kaolin particles are less toxic than microplastics. In general, at the same mass concentrations, the numerical concentrations of kaolin were much higher than those of all microplastics in our study. Kaolin impaired reproduction, *Daphnia* size and the day of first brood at much higher particle concentrations (4.75×10^9 particles/L) compared to microplastics. In line with our results, upon acute as well chronic exposure of *D. magna*, irregular microplastics had, respectively, a significant lower LC_{50} (PET; 5 μm; Gerdes et al., 2019) as well as $EC_{50\ Repro}$ (2.6 μm; Ogonowski et al., 2016) value than kaolin. This suggests (1) that the natural kaolin particle is less toxic than microplastics in daphnids and, (2) that the effect is independent of the mere number of particles.

Taken together, other factors than polymer type and numerical particle concentrations, that are specific to each plastic particle, influence adverse effects of microplastics. These may include physical characteristics, such as size, shape and surface morphology, and chemical characteristics, such as the presence of additives and side products.

4.2. Role of chemicals in microplastic toxicity

We aimed at elucidating whether plastic chemicals present in and leaching from the microplastics contribute to their toxicity. For that purpose, we compared within one microplastic type the chronic toxicity of the microplastics to that of particles without extractable chemicals, the chemicals extracted from the microplastics reflecting the chemicals that are used in plastic and can potentially be released in the environment under worst-case conditions. Additionally, we tested the chemicals migrating from plastic in aqueous medium within 21 days reflecting those realistically entering freshwater ecosystems.

Our results demonstrate that chemicals can be the main driver of microplastic toxicity. However, their contribution depends on the plastic type. For the PVC we analyzed, the extractable chemicals caused toxicity since only the plastic extract adversely affected *D. magna*. There was no toxicity when the chemicals were incorporated in the microplastics nor did the chemical toxicity migrate into aqueous medium over a 21-day period. This indicates that under more realistic conditions, the toxicity of leaching chemicals might be limited. However, the quantities and effects of chemicals leaching from plastic debris in natural environments are highly context dependent (e.g., type and surface area of debris, temperature, microbial activity) and difficult to generalize. In addition, it remains to be seen how the effects of chemicals leaching from artificially ground microplastics will translate to plastics aged in nature.

In contrast to PVC, the toxicity induced by the analyzed PUR and PLA was not caused by plastic chemicals since neither the extracted nor migrating compounds had negative impacts. Instead, the microplastics and extracted microplastics induced adverse effects implying that the particle characteristics of PUR and PLA microplastics are causative.

Few studies have compared the physical and chemical toxicity of microplastics. For instance, the negative impacts of PET fibers on survival of *D. magna* (Jemec et al., 2016) and PS beads on reproduction of *C. elegans* (Mueller et al., 2020) were not caused by their chemical leachates. In contrast, Oliviero et al. (2019) linked the toxicity of irregular PVC microplastics made from toys (<20 μm) on sea urchin to the leachable chemicals. Chemical-driven effects were also observed in plants. Here, leachates of polycarbonate (PC) granules but not whole microplastics affected germination of a garden cress (Pflugmacher et al., 2020). In contrast to our PUR particles that do not contain compounds toxic to *D. magna*, other PUR consumer products leached chemicals with acute toxicity to daphnids (Lithner et al., 2009). These studies strengthen the argument that chemicals can drive microplastic toxicity and clarify that the chemical toxicity is specific to the individual material and not necessarily to a polymer type. Nonetheless, there is some evidence that the toxicity of microplastics made of certain polymers, especially PVC and PC, is caused by the plastic chemicals.

In order to find out why only the plastic chemicals in PVC induced toxicity, we compared the chemical profiles of the three plastics (details in Zimmermann et al., 2019). Interestingly, the total abundance (peak area) was largest for the PLA extract followed by PVC and PUR extracts. Likewise, PLA contained 103 compounds, followed by PVC (52) and PUR (44). Thus, neither the abundance nor the number of plastic chemicals predicts the *in vivo* toxicity of plastic extracts observed in this study. We further prioritized the identified chemicals based on their abundance and *in vitro* toxicity and detected high priority chemicals in all three plastics, for instance the plasticizer tributyl acetylcitrate in PVC, the antioxidant butylated hydroxytoluene in PUR and the side product 9-octodecamide in PLA (Zimmermann et al., 2019). However, it still remains elusive whether the toxic effects of PVC on *D. magna* were caused by individual compounds or a mixture of chemicals. Overall, the chemicals inducing *in vivo* effects, likewise as the chemicals inducing *in vitro* toxicity, remain to be identified which makes further research necessary.

4.3. Role of physical characteristics in microplastic toxicity

The physical properties of microplastics, including size, shape, surface morphology and charge, may also play an important role in their toxicity. For instance, 100 nm PS beads were more toxic in *D. magna* than 2 μm PS beads (Rist et al., 2017) and PET fibers induced stronger effects than PE beads in *Ceriodaphnia dubia* (Ziajahromi et al., 2017). Regarding the surface charge, positively-charged amidine 200 nm PS nanobeads were more toxic than negatively charged carboxylated PS beads in *D. magna* (Saavedra et al., 2019). While identifying which physical property drives the toxicity of microplastics is not an easy task, this highlights that multiple factors need to be considered.

In terms of particle size, smaller microplastics did not induce a higher toxicity in our study: The adverse effects of PLA and PUR were induced by particles mostly smaller than 40 μm (MP and eMP) while the smaller PVC particles (mostly <20 μm) did not cause an effect. Compared to the suspension based on PVC microplastics, the one of extracted PVC contained much more small particles, probably as a consequence of fragmentation during extraction, but still was not toxic to *D. magna*. Due to technical limitations, we could not determine the occurrence of particles <2 μm. Thus, the contribution of smaller microplastics and nanoplastics potentially present in the suspensions and extracts remains unknown.

In terms of shape and surface morphology, we generated irregular microplastics from plastic consumer products. Since materials have different fragmentation pattern, creating identical particle shapes is not entirely feasible. Nevertheless, all selected microplastics share an irregular shape and rough surface. Here, PVC and PUR microplastics have a very similar, rounded shape but do not resemble each other with regards to their toxicity. *Vice versa*, PUR and PLA microplastics have a somewhat dissimilar shape but induced a comparable toxicity. Thus, shape is not the driving factor

for toxicity in our study. However, this may be different when investigating particles with more dissimilar shapes (e.g., beads vs. fibers).

Additionally, a higher numerical concentration at equal mass concentrations was not responsible for higher effects. For instance, PLA MP and eMP suspensions had lower numerical concentration than the PVC eMP suspension but PLA and not PVC particles affected life-history traits of *D. magna*. Thus, other particle-related differences of PLA compared to PVC microplastics, like the flatter and more angular shape or another surface charge of PLA, may render them more toxic. In general, the combination of the several physical characteristics specific to each particle type influences microplastic toxicity. This indicates the necessity to consider multiple physical properties of microplastics in future toxicity studies.

Summing up, for the microplastics we studied, the effects of PVC are driven by chemical toxicity while physical toxicity dominates for PUR and PLA microplastics. Concerning the latter, neither a higher numerical concentration, the specific particle size, shape nor surface morphology appears to be the sole relevant factor. Since PVC microplastics were still more toxic than PUR and PLA particles, chemicals seem to have a higher impact than physical properties on microplastic toxicity in our study.

4.4. Bioplastics are not necessarily safer than conventional plastics

Bioplastics are made from renewable resources (bio-based) and/or degrade in the natural environment by the action of microorganisms (biodegradable; Lambert and Wagner, 2017). They are especially prone to end up in natural ecosystems due to the promise that they easily degrade in nature which is often not even true (Haider et al., 2019). Although marketed as a more sustainable alternative, there are first indications from *in vitro* testing that they are not necessarily toxicologically safer than their petroleum-based counterparts (Zimmermann et al., 2019). Our *in vivo* results support that idea as PLA was more toxic than PVC and PUR with regards to daphnid mortality. Besides *D. magna*, also other aquatic organisms are susceptible to PLA microplastics. Exposure of the oyster *Ostrea edulis* to 0.8 or 80 μg/L of 65.6 mm (Green, 2016) and the lugworm *Arenicola marina* of 1.4–707 μm (Green et al., 2016) PLA microplastics resulted in elevated respiratory rates. While we cannot attribute the toxicity of the PLA to plastic chemicals in our study, PLA leachates induced *in vitro* baseline toxicity (Ramot et al., 2016; Zimmermann et al., 2019). This phenomenon is not limited to PLA but also applies to other bioplastics. For instance, aqueous leachates of polybutylene adipate terephthalate (PBAT) and polyhydroxybutyrate (PHB) granules increased the immobility of *D. magna* after 48 h of exposure (Göttermann et al., 2015). Taken together, bioplastics, like PLA, can be similarly toxic as conventional plastics and are especially prone to end up in the environment and therefore, might pose a particular hazard for aquatic organisms.

5. Conclusions

The aim of this study was to characterize the toxicity of microplastics from currently understudied materials as well as to elucidate whether the toxicity is driven by the chemicals present in microplastics. We, thus, chronically exposed *D. magna* to high concentrations of PVC, PUR and PLA microplastics or kaolin as well as to four exposure scenarios to differentiate between physical and chemical toxicity. The latter included untreated microplastics, microplastic particles without extractable chemicals as well as the compounds extracted or migrating from the plastics. All three microplastic types adversely affected the life history of *D. magna* at high concentrations. Here, the magnitudes of effect on multiple endpoints were material-specific with PVC being most toxic to reproduction and PLA inducing most mortality. We demonstrate that plastic chemicals are the main driver for toxicity in case of the PVC but not of the PUR and PLA microplastics investigated here. Additionally, the high mortality upon PLA exposure indicates that bioplastics can be similarly toxic as their conventional counterparts. Our findings highlight that microplastics cannot be treated as homogenous entity when assessing their environmental hazards. Instead, multiple plastic types as well as chemical compositions and physical characteristics of microplastics need to be taken into account. Importantly, studying the toxicity of other polymers than PS and PE, especially bioplastics, is particularly relevant.

CRediT authorship contribution statement

Lisa Zimmermann: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **Sarah Göttlich:** Formal analysis, Writing - review & editing. **Jörg Oehlmann:** Conceptualization, Writing - review & editing. **Martin Wagner:** Conceptualization, Writing - review & editing. **Carolin Völker:** Conceptualization, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115392.

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A5 Von der unsichtbaren zur durchschaubaren Verpackung. Prinzipien nachhaltiger Verpackungsgestaltung

Von der unsichtbaren zur durchschaubaren Verpackung. Prinzipien nachhaltiger Verpackungsgestaltung

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	LS	**LZ**	MB
Concept and design	50%	**30%**	20%
Conducting tests and experiments	34%	**33%**	33%
Compilation of data sets and figures	34%	**33%**	33%
Analysis and interpretation of data	34%	**33%**	33%
Drafting of manuscript	50%	**25%**	25%

Prinzipien nachhaltiger Verpackungsgestaltung

Von der unsichtbaren zur durchschaubaren Verpackung

Meeresmüll und Mikroplastik sind allgegenwärtig und in aller Munde, gleichzeitig bleibt Verpackung in vielfacher Hinsicht unsichtbar und schweigsam. Ein nachhaltiger Umgang mit Verpackung muss diese in ihren Inhaltsstoffen und Umweltauswirkungen durchschaubar und damit diskutierbar machen. Von Lukas Sattlegger, Lisa Zimmermann und Maik Birnbach

Als Medium, das einerseits Aufmerksamkeit wecken, andererseits aber nicht vom eigentlichen Produkt ablenken, sondern diesem Prominenz geben soll, hat die Verpackung eine ambivalente Beziehung zu Transparenz (Cochoy 2000). Die Verpackung klärt über das verpackte Produkt auf und macht dieses dadurch durchschaubar (z. B. durch Siegel und Produktinformationen), während sie sich gleichzeitig selbst „unsichtbar" macht. Diese Unsichtbarkeit ist sowohl im wörtlichen Sinn zu verstehen, etwa bei der Nutzung von Glas und durchsichtigen Kunststoffen als beliebte Verpackungsmaterialien, die das Produkt in den Fokus rücken. Unsichtbar wird Verpackung aber auch im metaphorischen Sinn, da die Verpackung und ihre Eigenschaften meist nicht Kommunikationsinhalt, sondern nur Kommunikationsmedium ist.

Diese Unsichtbarkeit von Verpackung wandelt sich jedoch, wenn sie nach ihrer Nutzungsphase zum Verpackungsmüll wird. Insbesondere als Müll in Ozeanen, an Stränden und in unseren Städten wird Verpackung sichtbar und zum Gegenstand der gesellschaftlichen Auseinandersetzung. Verpackung wird damit von einer unhinterfragten Technologie zur Organisation von Märkten zu einem symptomatischen Objekt eines Umweltproblems (Hawkins 2012).

Ein anderen Umgang mit Verpackung ist nötig

Zur Lösung dieser mit Verpackung assoziierten Nachhaltigkeitsprobleme braucht es eine Transformation im Umgang mit Verpackungen und ihrer Unsichtbarkeit. Die Verpackung muss für Nutzende durchschaubar werden. Das betrifft ihre ganze Produktionskette, vom Rohmaterial über die Folie bis hin zur fertigen Verpackung. Verpackung muss an Kriterien gemessen werden, die über Preis, Ästhetik und technische Funktionalität hinausgehen und Fragen von Kreislauffähigkeit, Umwelt- und Gesundheitsverträglichkeit miteinbeziehen (EPEA 2019). Die Thematisierung von Verpackung darf sich nicht auf Symptome einer unsachgemäßen Nutzung beziehungsweise Entsorgung beschränken, sondern muss Umwelt- und Gesundheitsverträglichkeit als Teil ihrer Produktion und Nutzung in den Fokus nehmen. Dazu müssen wir die Gründe für die verpackungsbezogene Unsichtbarkeit genauer erörtern. Wir bringen als Ökotoxikologin, die sich mit Inhaltsstoffen von Kunststoffverpackungen beschäftigt, Nachhaltigkeitsmanager, der Verpackungen konzipiert, und Sozialwissenschaftler, der den Umgang mit Verpackungen im Lebensmittelsystem beforscht, je unterschiedliche Perspektiven und Erfahrungen in den Diskurs ein.

In diesem Beitrag loten wir gemeinsam Möglichkeiten aus, wie die „unsichtbare" Verpackung sichtbar und folglich durchschaubar gemacht werden kann. Dazu stellen wir Beispiele aus unserem Forschungs- und Unternehmensalltag vor, an denen wir selbst auf „Unsichtbarkeiten" und damit Undurchschaubarkeiten von Verpackung gestoßen sind. Lisa Zimmermann, Ökotoxikologin, beschreibt das fehlende Wissen und den mangelnden Wissenstransfer über die chemische Zusammensetzung von Kunststoffverpackungen und die damit einhergehenden Schwierigkeiten, ihre gesundheitliche und ökologische Unbedenklichkeit zu bewerten. Dann beleuchtet Maik Birnbach, Nachhaltigkeitsmanager bei *einhorn* (einhorn products GmbH), wie mangelndes Wissen über Produktanforderungen und Zusatzstoffe nachhaltiges Verpackungsdesign beeinträchtigen. Lukas Sattlegger, Soziologe, beschreibt die Schwierigkeit, wissenschaftliche Nachhaltigkeitsbewertungen in unternehmerische Entscheidungen zu übersetzen. Auf Basis dieser Erfahrungsberichte geben wir abschließend Handlungsempfehlungen für das Gelingen einer sichereren und nachhaltigen Verpackungsgestaltung, aber auch die Zusammenarbeit relevanter Akteure.

Die Intransparenz der chemischen Zusammensetzung

Ziel meiner (Lisa Zimmermann) derzeitigen ökotoxikologischen Forschungsarbeiten ist es, die Chemikalienmischung in Kunststoffverpackungen ausführlich zu charakterisieren (Zimmermann et al. 2019). Als Untersuchungsobjekte habe ich Verpackungen aus verschiedenen Kunststoffarten gewählt. Das bedeutet, sie basieren auf verschiedenen Polymertypen (z. B. Polyethylen und Polystyrol). Kunststoffe enthalten daneben zahlreiche weitere Substanzen, wie Füllstoffe und Zusatzstoffe (z. B. Weichmacher, Antioxidantien, Stabilisatoren, Farb-

stoffe), die dem Material die gewünschten Eigenschaften verleihen (Muncke 2009). Die chemische Zusammensetzung von Kunststoffprodukten ist dabei so vielfältig wie ihre Anwendungen. Während einzelne Substanzen, wie beispielsweise Bisphenol A, ausführlich untersucht sind, finden die meisten weiteren sowie deren Mischungen mit anderen Substanzen im jeweiligen Endprodukt wenig Beachtung und somit ist auch ihre Sicherheit für Mensch und Umwelt nicht garantiert (Wagner 2017).

Mich interessierte, ob einige Kunststofftypen unbedenklicher sind als andere und ob sich dies auf den jeweiligen enthaltenen Chemikalienmix zurückführen lässt. In meinen Untersuchungen löste ich dazu die Chemikalienmischung aus den unterschiedlichen Kunststoffen heraus und untersuchte anhand von Labortests die negativen Effekte dieser Mischung unter anderem auf menschliche Zellen. Zudem analysierte ich die chemische Zusammensetzung der Kunststoffe. In den Forschungsarbeiten galt mein besonderes Interesse den sogenannten Biokunststoffen, die entweder biobasiert, das heißt aus nachwachsenden Rohstoffen hergestellt, und/oder bioabbaubar sind, das heißt in der Anwesenheit von Mikroorganismen zersetzt werden (DIN 2011). Da sie als nachhaltigere Alternative zu erdölbasierten, nicht bioabbaubaren Kunststoffen vermarktet werden, wollte ich herausfinden, ob sie eine unbedenklichere Alternative hinsichtlich der in ihnen enthaltenen Chemikalien darstellen. Um meine Untersuchungen durchführen zu können, musste ich den Polymertyp (z. B. Cellulose, Stärke, Polymilchsäure) kennen, auf dem die Produkte basierten. Zudem versuchte ich, Informationen zu den weiteren chemischen Inhaltsstoffen zu bekommen. Hierbei offenbarten sich Intransparenz und Wissenslücken, die einen toxikologischen Bewertungsprozess erschweren.

Bei meiner Produktakquise im Lebensmitteleinzelhandel stellte ich fest, dass sich auf den meisten Produkten aus Biokunststoffen kein Hinweis auf deren Polymertyp befindet. Während auf vielen erdölbasierten Produkten aus gängigen Polymertypen dieser über einen Recyclingcode kenntlich gemacht wird (z. B. Nummer 1 = Polyethylenterephthalat (PET)), existiert für bioabbaubare Kunststoffe kein solches Kennzeichnungssystem. Lediglich Vermerke wie „biobasiert"/„aus nachwachsenden Rohstoffen hergestellt" oder „kompostierbar" weisen auf ihren Ursprung und ihre Abbaubarkeit hin. Zudem ist statt der Angabe der vollständigen Inhaltsstoffe eines Kunststoffproduktes lediglich vermerkt, welche einzelnen Substanzen sich *nicht* in dem Produkt befinden („ohne Bisphenol A", „aluminiumfrei"). Die Hersteller sind nicht verpflichtet, die genaue chemische Zusammensetzung eines Kunststoffproduktes offenzulegen. Somit bleibt diese auch für Konsument/innen und Entsorgungsunternehmen undurchschaubar. Um dennoch detailliertere Angaben zu Polymertyp und chemischer Zusammensetzung zu bekommen, habe ich versucht, die Akteure der Produktionskette der ungefähr 50 untersuchten Artikel zu kontaktieren. Zunächst stellte sich die Identifikation aller an Herstellung und Vertrieb eines Produktes beteiligten Akteure als schwierig heraus, da zum Beispiel Unternehmen verkauft werden oder Vertreiber keine Auskunft zu ihren Herstellern geben wollten. Es ist also bereits schwer durchschaubar, wer überhaupt im Besitz von relevantem Wissen ist. Zudem sind Akteure oft nicht gewillt ihr Wissen transparent zu machen. So blieben Antworten auf meine Nachfragen entweder ganz aus oder ich bekam lediglich unvollständige Informationen hinsichtlich der chemischen Zusammensetzung der Verpackungen. Dieser mangelnde Wissenstransfer existiert ebenso zwischen den Akteuren des Herstellungsprozesses, sodass selbst bei diesen Wissenslücken hinsichtlich der Produktzusammensetzungen bestehen. Die Intransparenz ergibt sich also zum einen aus mangelnder Weitergabe von Informationen in der Produktionskette. Zum anderen entstehen aber auch im Produktionsprozess unbeabsichtigt neue Substanzen, die allen Akteuren unbekannt sind. Diese umfassen beispielsweise Neben- oder Abbauprodukte, die erst während des Prozessierens eines Kunststoffproduktes entstehen (Muncke 2009). Dementsprechend kennt selbst der Hersteller eines (Zwischen-)Produkts nicht alle Chemikalien, die in diesem enthalten sind.

Für mich als Wissenschaftlerin ergibt sich ein weiteres Problem: Um eine Bandbreite verschiedener Kunststoffarten untersuchen zu können, wandte ich mich neben der Akquise im Einzelhandel für spezifische Produkte an diverse Hersteller. Diese verkaufen ihre (Zwischen-)Produkte oft entweder gar nicht an Universitäten oder eine Materialübertragungsvereinbarung schreibt vor, dass das Materialmuster lediglich auf seine Performanz, nicht aber auf seine Zusammensetzung überprüft werden dürfe. Es wird also aktiv verhindert, dass Dritte selbst Wissen generieren können, was eine adäquate Bewertung der Auswirkungen dieser Produkte auf Mensch und Umwelt verhindert.

Kunststoffartikel mit Lebensmittelkontakt unterliegen einer gesonderten EU-Richtlinie, die garantieren soll, dass diese gesundheitlich unbedenklich sind. Allerdings sieht selbst diese nur die Bewertung des Übertretens einzelner, bekannter Substanzen in Nahrungsmitteln vor (Europäische Kommission 2011). Gesundheitliche Auswirkungen von „Unbekannten" sowie der Substanzmischung, welche beispielsweise im fertigen Verpackungsmaterial vorhanden ist und zum Teil in die darin verpackten Lebensmittel übertreten kann, müssen nicht untersucht werden. Somit ist auch hier die Unbedenklichkeit der chemischen Inhaltsstoffe für Mensch und Umwelt nicht garantiert. Dies haben auch meine Untersuchungen bestätigt. Ein Großteil der untersuchten Produkte enthielt bedenkliche Substanzmischungen, die sich erstens nicht vollständig identifizieren ließen und zweitens negative Effekte in Zelltests zeigten (Zimmermann et al. 2019).

Fehlendes Wissen zu Produktanforderungen und Recyclingfähigkeit

Die Undurchschaubarkeit der Verpackung behindert neben toxikologischen Bewertungen auch die Entwicklung nachhaltigerer Verpackungsdesigns. Eine nachhaltige Verpackung ist

„Ökobilanzen sind grundsätzlich extrem kontextabhängig und können meist nicht in verallgemeinerbare Richtlinien übersetzt werden."

nach meinem (Maik Birnbach) Verständnis möglichst gering bis nicht existent (Reduce bzw. Zero Waste), schützt das Produkt, wenn nötig, ist mehrfach nutzbar (Reuse-Konzepte, wie z. B. die Mehrwegflasche) oder nutzt zumindest kreislauffähige Materialien (Recycling), die ökologisch vorteilhaft produziert wurden. Der Komplexität dieser Kriterien steht jedoch der unvollständige Zugang zu den dafür notwendigen Informationen gegenüber: Um welches Material handelt es sich? Ist es notwendig für den Produktschutz? Wie wurde es hergestellt und aus welchen Ressourcen? Wie gut kann es tatsächlich wiederverwendet werden und ist der Weg zur Verwertung auch für Nutzende nachvollziehbar? Schlussendlich: Welchen Fußabdruck hat diese Verpackung im Vergleich zu einer Alternative? All dies sind Informationen, die man zu häufig nicht einfach von Lieferanten wie Verpackungsherstellern oder Materialproduzenten erhält. Im Folgenden führe ich zwei Beispiele solcher Wissens- und Kommunikationsmängel aus, die mir als Verantwortlicher für nachhaltige Verpackungen bei *einhorn* begegnet sind:

Kondome sollen nach *ISO-Norm 4074:2017* mit einem Schutz gegen das Austreten des Gleitmittels und das Eindringen von Licht und Sauerstoff ausgestattet werden. Da die Norm selber jedoch keine Barriere-Grenzwerte vorgibt (Wie stark muss der Schutz zum Beispiel gegen Sauerstoff tatsächlich sein, um das Kondom zu schützen?), hat sich als Industriestandard durchgesetzt, ein Verbundmaterial aus Plastik- und Aluminiumfolie (oder eine Aluminiumbedampfung) einzusetzen, da Aluminium gemeinhin als stärkste Barriere gegen Gas- oder Lichtdurchtritt gilt. So wichtig der Produktschutz (insbesondere in diesem Fall) ist, so wichtig scheint vor unseren globalen Herausforderungen jedoch auch die Frage: Schlagen hier Produktschutz und Haltbarkeit (bei Kondomen immerhin ca. drei bis fünf Jahre) die Nachhaltigkeit der Verpackung? Es handelt sich nicht nur um sorglosen Ressourcenverbrauch (Einsatz von Aluminium), sondern auch um die Erzeugung einer Verpackung, die bei heutigem Stand der Technik nicht wiederverwertet werden kann, da sich der Materialverbund nicht auftrennen lässt. Zudem zeigt die Ökobilanz eines Kondoms, dass der Einsatz einer Aluminiumfolie in der Verpackung für einen großen Teil des Fußabdrucks verantwortlich ist (Birnbach et al. 2020). Wie sieht es in diesem Zusammenhang mit Lebensmittelverpackungen aus? Warum können beispielsweise manche Teesorten in einer Papp-Box verkauft werden, während es für andere Hersteller notwendig erscheint, eine Aluminiumbarriere in ihrer Verpackung zu verarbeiten? Meine Erfahrung zeigt, dass Verpackungsmittelhersteller und Marken viel zu selten wissenschaftliche Antworten auf diese Fragen haben und schlicht ihrer Erfahrung oder einem „das machen wir schon immer so" oder „das machen doch alle so" trauen. Bisher ist zu häufig unklar, welchen Schutz die Verpackung eigentlich tatsächlich bieten muss. Das Wissen darüber ist jedoch essenziell für ein nachhaltiges Verpackungsdesign.

In puncto Kreislaufwirtschaft ist noch ein weiteres Beispiel interessant: Farben, Kleber und weitere Zusatzstoffe. All diese Materialkomponenten haben einen Einfluss darauf, wie und ob sich Verpackungen sortieren und recyceln lassen und in welcher Qualität der recycelte Rohstoff am Ende vorliegt. So weist beispielsweise die *International Association of the Deinking Industry* (INGEDE) darauf hin, dass sich vernetzende Farben und Öle (UV-Farben, Farben auf Sojaöl-Basis, teils auch Flüssigtoner) im Papier-Recyclingprozess nicht mehr von Papierfasern lösen lassen und so die Fasern nicht mehr in Recyclingpapier mit hoher Qualität verwendet werden können (INDEGE 2019). Die Auswahl der Druckfarben und Techniken hat also eine nicht zu vernachlässigende Rolle bei der Bewertung der Recyclingfähigkeit und letztlich der Nachhaltigkeit von Verpackungen (Ähnliches gilt bspw. auch für die Sortierfähigkeit von schwarz eingefärbten Plastikverpackungen). Interessant sind dabei zwei Aspekte: Erstens muss die Recyclingfähigkeit bisher nicht verpflichtend getestet werden, obwohl Testverfahren zur Verfügung stehen. Zudem werden Testergebnisse von den entsprechenden Institutionen nicht veröffentlicht, obwohl dies die Auswahl der Farb- und Drucksysteme für Verpackungsdesigner und Drucker vereinfachen würde. Zweitens werden unter anderem Druckfarben in Ökobilanzen explizit ausgeschlossen, da sie einen zu geringen Anteil an der Masse der Verpackung ausmachen. So wird in gängigen Ökobilanzen für Getränkekartons die Druckfarbe vernachlässigt, weil sie weniger als 1 % des Gewichts des Getränkekartons ausmacht (Kauertz et al. 2018). Dabei können Druckfarben und Zusatzstoffe auch toxische Substanzen (bspw. Schwermetalle) enthalten. Werden diese nicht während des Recyclings entfernt, werden recycelte Materialien verunreinigt und so gesundheitlich bedenklich (EPEA 2018). Das systematische Fehlen solcher Detailinformationen zu Zusatzstoffen und ihren Auswirkungen erschwert die Entwicklung und Auswahl einer nachhaltigen, gesundheitlich unbedenklichen und kreislauffähigen Verpackung.

Hindernisse in unternehmerischen Entscheidungsprozessen

Mangelhafter Wissenstransfer ist nicht nur innerhalb der Versorgungskette und gegenüber der Öffentlichkeit und Wissenschaft ein Problem. Eine eingeschränkte Wissensvermittlung ist auch von der Wissenschaft in die Wirtschaft zu beobachten. Die Schwierigkeit, wissenschaftliches Systemwissen in

konkrete Handlungskriterien zu übersetzen, konnte ich (Lukas Sattlegger) als Sozialwissenschaftler im Zuge meines Forschungsaufenthalts in den wöchentlichen Sortimentsbesprechungen beim Bio-Großhandel *Kornkraft Naturkost* beobachten. Schon in einem unserer ersten Gespräche während meiner Zeit bei Kornkraft machte mich die Geschäftsführerin mit der Idee vertraut, bei der Sortimentszusammenstellung das Kriterium „Nachhaltigkeit von Verpackung" stärker zu berücksichtigen. Meine Aufgabe als Wissenschaftler war, dabei zu helfen, Kriterien für „gute" und „schlechte" Verpackungen zu entwickeln und eine Entscheidungshilfe für die Produktauswahl zu definieren. In den Sortimentsbesprechungen könnten diese Kriterien dann in Entscheidungsprozesse einfließen. Im Zuge dieser Besprechungen kristallisierten sich einige Wissensdimensionen heraus, die eine Übertragung von wissenschaftlichem Wissen in konkrete unternehmerische Entscheidungskompetenz erschweren. Die verfügbaren wissenschaftlichen Bewertungskriterien erwiesen sich in der unternehmerischen Praxis als oftmals undurchschaubar und schwer anwendbar.

Ökobilanzen sind grundsätzlich extrem kontextabhängig und können meist nicht in verallgemeinerbare Richtlinien übersetzt werden, wie der Wunsch der *Europäischen Kommission* nach mehr Vergleichbarkeit von Ökobilanz-Ergebnissen zeigt (UBA 2018). Das erschwert das Finden einfacher, kommunizierbarer Entscheidungskriterien. Für Praxisakteure ist diese Komplexität oft nicht durchschaubar, das Wissen bleibt abstrakt. Mehrmals werde ich als Wissenschaftler mit konkreten Fragen konfrontiert, was denn jetzt besser sei – diese oder jene Verpackung. Fragen, die ich durch Verweis auf Forschungsbedarf und Kontexte *(„das hängt vom Entsorgungs- und Verwertungssystem ab")* nicht zufriedenstellend beantworten konnte. Die Wissenschaft tut sich aufgrund der Komplexität und Dynamik der Rahmenbedingungen schwer, den unternehmerischen Wissensbedarf nach klaren und durchschaubaren Kriterien adäquat zu bedienen. Auch wenn die Ökobilanzen immer besser und realistischer werden, sie können nur konkrete Verpackungen unter aktuellen Kontextbedingungen vergleichen, aber keine allgemeingültigen Anleitungen zur Verpackungsauswahl liefern.

Wissenschaftliche Nachhaltigkeitsbewertungen sind für Praxisakteure nicht nur schwer verständlich, sondern auch teilweise inkompatibel mit der unternehmerischen Entscheidungspraktik. Die Sortimentsgespräche bei *Kornkraft* sind von einer Kultur des Diskurses und der gemeinsamen Abwägung von Vor- und Nachteilen getragen. Die Art des Wissens und der Kompetenzen ist multidimensional und unterscheidet sich stark von naturwissenschaftlicher Exaktheit. Erfahrungswissen, subjektive Gefühle und Geschmacksproben lassen sich nicht einfach in harte Kriterien übersetzen beziehungsweise diesen gegenüberstellen. Auch die Bewertung von Nachhaltigkeit und Verpackung wird in diesen Gesprächen anhand unterschiedlicher Gesichtspunkte betrachtet und dreht sich neben der wissenschaftlichen Ökobilanz auch um die Rezeption und Wahrnehmung durch Kund/innen. Eine systematische Integration und Gewichtung rein rationaler wissenschaftlicher Kriterien in diese Besprechungssituationen fällt daher schwer und würde die Entscheidungsdiskurse womöglich irritieren, selbst wenn es gelingen sollte, klare Bewertungskriterien zu entwickeln. Diese müssten sich eben nicht in mathematischen Modellen, sondern in der diskursiven Aushandlungspraxis der Besprechungen bewähren und von den Akteuren kompetent angewandt werden.

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Eine weitere Schwierigkeit der ökologisch motivierten Entscheidungsfindung liegt in der Einbettung konkreter Entscheidungspraktiken in ökonomische Logik und Systeme. Verpackung und Nachhaltigkeit sind in der unternehmerischen Sortimentsentscheidung nur zwei Kriterien unter vielen. Sie stehen in Konkurrenz zu anderen Prioritäten und sind dabei unter anderem der Gewinnlogik unterstellt: Unternehmen sind in ein Wirtschaftssystem eingebunden und agieren darin nicht unabhängig von anderen Akteuren, gerade auch in Bezug auf Nachhaltigkeitsvorgaben. Der praktische Einfluss dieser Verknüpfungen zeigt sich etwa im Ideal der Wahlfreiheit der Konsumierenden *(„sonst kommt er nicht nochmal")*, als auch im Vergleich mit anderen Unternehmen *(„aber wenn, dann müsste Kriterienkatalog für nachhaltige Verpackung vom Verband kommen und schon bundesweit sein, sonst bringt es nichts")*. Nachhaltige Alternativen müssen also nicht nur in ihren ökologischen, sondern auch in ihren sozialen und ökonomischen Folgen und Chancen durchschaubar sein, um sich zu bewähren. Dieser Bezug zu ökonomischen und sozialen Nachhaltigkeitskriterien zeigt die Herausforderung einer transparenteren Nachhaltigkeitsbewertung von Verpackung.

Transparenz und Wissensaustausch einfordern und praktizieren

Die Unterschiedlichkeit der Perspektiven und Beispiele zeigt, dass die Undurchschaubarkeit von Verpackung mehrere Gründe auf verschiedenen Ebenen hat: Mal fehlt es an Wissen, mal wird vorhandenes Wissen verschwiegen (Intransparenz), mal ist verfügbares Wissen nicht anwendbar oder anschlussfähig (Inkompatibilität). Trotz der Vielschichtigkeit der beschriebenen Probleme, lassen sich daraus zentrale Prinzipien ableiten, die kollaborative und nachhaltige Verpackungsgestaltung begünstigen und diese zu etwas Durchschaubarem

und damit auch (öffentlich) Verhandelbarem machen. Die folgenden Handlungsempfehlungen richten sich gleichermaßen, aber mit unterschiedlichen Schwerpunkten an politische Entscheidungsträger/innen, wirtschaftliche Akteure und Nachhaltigkeitsforschende:

- Positivlisten in der Materialzulassung: Konsequent für sichere Materialien und eine funktionierende Kreislaufwirtschaft wäre der Einsatz definierter zugelassener Materialien und Substanzen. So könnten Positivlisten (*white lists*) Stoffe auflisten, die verwendet werden dürfen. Sie könnten bisher gängige Negativlisten (*black lists*) mit verbotenen oder begrenzt zu verwendenden Substanzen ersetzen.
- Transparenz bezüglich Inhaltsstoffe: Inhaltsstoffe und Materialinformationen sollten möglichst weitgehend zugänglich gemacht werden. Wo das Ideal einer grundsätzlichen Offenlegung von chemischen Zusammensetzungen aufgrund von Wettbewerbsnachteilen nur eingeschränkt möglich ist, sollten die Inhaltsstoffe und ihre Unbedenklichkeit von unabhängigen Institutionen bewertet werden, wie es etwa bei der *Cradle-to-cradle*-Zertifizierung praktiziert wird (EPEA 2019). Diese Transparenz muss auch für den Einsatz von Zusatzstoffen wie Druckfarben und Kleber gelten.
- Erweiterte Materialtests: Um auch unbekannte Substanzen, die etwa im Herstellungsprozess entstehen, zu berücksichtigen und die Unbedenklichkeit möglicher Mischeffekte zu garantieren, könnten toxikologische Tests eingesetzt werden. Hier wird die Chemikalienmischung in jedem Kunststoffprodukt schon während der Herstellung untersucht (Groh 2017; Zimmermann et al. 2019). Auch die Recyclingfähigkeit von Verpackungen sollte vor der Anwendung verpflichtend getestet werden. Bereits heute könnten verantwortungsvolle Händler entsprechende positiv bewertete Materialgesundheits- und Recyclingtests von ihren Industriepartnern einfordern und zum Einkaufskriterium neben ökonomischen Faktoren machen.
- Kultur der Kollaboration: Allgemeiner, aber nicht weniger wichtig ist die Etablierung einer Kultur der Kollaboration. Wo heute zwischen Unternehmen, Wissenschaft und Öffentlichkeit fehlende Kommunikation (zuweilen aufgrund von Geschäftsgeheimnissen, Konkurrenz, Misstrauen oder fehlender Priorität) herrscht, braucht es Wissensaustausch und Zusammenarbeit. Das betrifft insbesondere die Zusammenarbeit innerhalb der Produktionskette zwischen Kunststoffherstellern, Verpackungsherstellern, Produkterzeugern und Handel.
- Problemorientierter Wissenstransfer: Wissenschaftliche Forschung zur Nachhaltigkeitsbewertung von Verpackungen muss den Dialog mit Unternehmen, Politik und Öffentlichkeit verstärken und dabei helfen, existierende Erkenntnisse in anwendbares Orientierungswissen zu übersetzen. Es braucht einen problemorientierten Wissenstransfer, der es ermöglicht, verfügbares Nachhaltigkeitswissen breit verstehbar und nutzbar zu machen und bestehende Wissenslücken besser zu identifizieren.

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A6 Zusammenfassung

Kunststoffprodukte haben sich in den letzten 70 Jahren zu einem integralen Bestandteil des alltäglichen Lebens entwickelt, haben Fortschritt gebracht und die Lebensqualität verbessert. Ihr vielfältiger Einsatz wird durch eine ebenso vielfältige Zusammensetzung ermöglicht. Je nach Anwendung bestehen Kunststoffprodukte aus unterschiedlichen Polymeren, wie Polystyrol (PS) oder Polyvinylchlorid (PVC), und Additiven, wie Weichmachern, Farbstoffen und Flammschutzmitteln. Neben diesen absichtlich zugesetzten Chemikalien sind im Endprodukt unbeabsichtigt eingebrachte Substanzen, wie Nebenprodukte, Abbauprodukte und Verunreinigungen enthalten. Über 4 000 Chemikalien werden allein mit Kunststoffverpackungen in Verbindung gebracht. Bisher sind allerdings nur wenige Inhaltsstoffe überhaupt untersucht und toxikologisch charakterisiert. Beispiele hierfür sind Bisphenol A oder Phthalate, von denen einige aufgrund ihrer gesundheitlichen Auswirkungen entweder bereits verboten sind oder freiwillig nicht mehr in Kunststoffen verwendet werden. Daher ist unklar, ob Kunststoffprodukte als solche, d.h. mit all ihren Inhaltsstoffen, toxikologisch unbedenklich sind. Laut derzeitig geltenden Regularien ist beispielsweise in der Europäischen Union für Kunststoffe mit Lebensmittelkontakt nur die Untersuchung der wissentlich zugesetzten Chemikalien als Einzelsubstanz vorgeschrieben. Dieser Ansatz vernachlässigt sowohl die Wirkung unbekannter Substanzen sowie die im Endprodukt vorliegende Mischung an Chemikalien.

Vor diesem Hintergrund soll die vorliegende Arbeit zur toxikologischen Charakterisierung von Kunststoffprodukten beitragen. Der Fokus liegt dabei auf den Chemikalien, welche in Kunststoffen enthalten sind und daraus auslaugen können. Damit bildet die Arbeit eine wichtige Grundlage für die weitergehende Beurteilung der gesundheitlichen Auswirkungen von Kunststoffen.

Die erste Studie dieser Dissertation (A1) nahm alltägliche Kunststoffprodukte in den Blick. Hierfür wurden 34 Produkte aus den erdölbasierten Polymertypen HDPE, LDPE, PP, PS, PET, PVC und PUR sowie dem biobasierten und bioabbaubaren PLA ausgewählt und die darin enthaltenen Chemikalien mit Methanol extrahiert.[21] Die gewonnenen Extrakte wurden in vier In-vitro-Assays auf unspezifische Toxizität und endokrine Aktivität untersucht sowie mit hochauflösender non-target Gaschromatographie-Massenspektrometrie auf ihre chemische Zusammensetzung analysiert. Der Großteil der Produkte (74%) enthielt toxische

[21] Die Abkürzungen der untersuchten Kunststoffe stehen für High density polyethylen (HDPE), Low density polyethylen (LDPE), Polypropylen (PP), Polyethylenterephthalat (PET) und Polyurethan (PUR).

Chemikalienmischungen. Dabei induzierten 62% der Extrakte eine unspezifische Toxizität, 41% eine oxidative Stressantwort, 12% eine estrogene und 27% eine antiandrogene Wirkung. In den Kunststoffprodukten befanden sich insgesamt mehr als 1 400 semi-volatile Chemikalien, wovon sich der Großteil (> 80%) mittels chemischer Analytik nicht identifizieren ließ und somit unbekannt ist. Identifizierte Substanzen umfassten Additive, Monomere sowie unbeabsichtigt eingebrachte Substanzen. Die Ergebnisse verdeutlichen, dass Alltagsprodukte aus Kunststoff toxische Chemikalienmischungen enthalten können, die sich aus einer Vielzahl meist unbekannter Chemikalien zusammensetzen.

Biokunststoffe, d.h. aus nachwachsenden Rohstoffen hergestellte (biobasierte) und/oder unter bestimmten Bedingungen biologisch abbaubare (bioabbaubare) Kunststoffe, sowie pflanzenbasierte Kunststoffe, die aus natürlichen Polymeren wie Stärke bestehen, werden als nachhaltigere Alternative zu konventionellen, erdölbasierten Kunststoffen angesehen und beworben. Allerdings ist ungeklärt, ob diese aus toxikologischer Sicht auch als unbedenklicher einzustufen sind. Deshalb untersuchte die zweite Studie (A2) 43 Produkte aus Bio-PE und Bio-PET (biobasiert), PBS und PBAT (bioabbaubar), PLA und PHA (biobasiert und bioabbaubar) sowie Mischungen basierend auf Stärke, Cellulose und Bambus (pflanzenbasiert).[22] In den In-vitro-Tests hatten 67% der Proben auf mindestens einen der vier betrachteten Endpunkte eine negative Auswirkung. Die Höhe der ausgelösten Effekte war dabei vergleichbar zu der von konventionellen Kunststoffen. Daneben zeigte die non-target Flüssigchromatographie-Massenspektrometrie, dass 80% der untersuchten Extrakte mehr als 1 000 nicht-volatile Substanzen enthielten, die größtenteils spezifisch für das jeweilige Produkt waren. Diese Ergebnisse belegen, dass sich biobasierte und bioabbaubare Materialien hinsichtlich ihrer Toxizität und chemischen Komplexität kaum von herkömmlichen Kunststoffen unterscheiden.

Da Mensch und Umwelt nur gegenüber diesen Chemikalien exponiert sind, wenn sie unter realen Bedingungen aus dem Kunststoff freigesetzt werden, wurden die Chemikalien in der dritten Studie (A3) anders als in Studie 1 und 2 nicht mit Hilfe von Methanol extrahiert, sondern mit Wasser aus den Produkten gelöst (= Migrate). Hierfür wurden die 24 Produkte ausgewählt, die in der ersten Studie Toxizität induziert hatten. In den In-vitro-Tests bewirkten alle wässrigen Migrate eine unspezifische Toxizität, und 13 der untersuchten Proben inhibierten den humanen Androgenrezeptor. Die chemische Analytik zeigte, dass sich zwischen 1 und 84% der Chemikalien eines Kunststoffprodukts im Wasser nachweisen

[22] Die Abkürzungen stehen für Polybutylensuccinat (PBS), Polybutyrat-Adipat-Terephthalat (PBAT) sowie Polyhydroxyalkanoate (PHA).

ließen. Dies entspricht einer Anzahl von 17 bis 8 936 nicht-volatilen Substanzen. Rund 97% der detektierten Chemikalien sind unbekannt. Die Untersuchungen verdeutlichen, dass auch unter realen Bedingungen deutlich mehr Chemikalien aus Kunststoffen auslaugen als bisher bekannt. Zudem können diese Substanzmischungen bereits in niedrigen Konzentrationen Effekte in den In-vitro-Assays auslösen. Dies lässt Zweifel an der gesundheitlichen Unbedenklichkeit von Kunststoffprodukten aufkommen.

Die Ergebnisse der drei Studien verdeutlichen, dass Plastik nicht gleich Plastik ist. So zeichnet sich jedes Produkt durch eine individuelle chemische Zusammensetzung und In-vitro-Toxizität aus. PVC, PUR sowie Stärke- und Cellulose-Mischungen enthielten tendenziell mehr Chemikalien, die gleichzeitig eine höhere Toxizität besaßen, während PET, HDPE sowie Bio-PE tendenziell weniger Substanzen von insgesamt geringerer Toxizität enthielten. Allerdings umfasste jeder der getesteten Materialtypen mindestens ein toxisches Produkt.[23] Gleichermaßen besaßen untersuchte Materialien mit Lebensmittelkontakt zwar meist eine geringere Toxizität als die ohne Lebensmittelkontakt, dennoch gab es auch hier Ausnahmen. Über den Herstellungsprozess vom Ausgangsmaterial (Plastikpellets) zum Endprodukt scheint die Toxizität und Anzahl an Substanzen generell zuzunehmen. Allerdings können auch einzelne Rohmaterialien bereits toxische Chemikalien enthalten.

Wenn Kunststoffe in die Umwelt gelangen, fragmentieren sie über die Zeit zu immer kleineren Partikeln. Aufgrund der geringen Größe kann sogenanntes Mikroplastik (< 5 mm) von einer Vielzahl von Organismen aufgenommen werden. Ebenso wie Kunststoffprodukte, besitzen auch kleine Plastikfragmente eine komplexe chemische Zusammensetzung. Über die chemischen Eigenschaften hinaus unterscheidet sich Mikroplastik zudem in seinen physikalischen Eigenschaften, wie Größe, Form, Farbe und Oberflächenbeschaffenheit. Bisher durchgeführte Effektstudien bilden diese Heterogenität des in der Umwelt vorkommenden Mikroplastiks nur unzureichend ab. Sie beschränken sich meist auf einzelne Polymertypen, gehen selten auf die Chemikalien in dem Material ein und betrachten oft nur runde Partikel, obwohl Fragmente in der Umwelt überwiegen. Des Weiteren werden im Versuchsdesign oft Kontrollen mit natürlich vorkommenden Partikeln wie Kaolin vernachlässigt, die untersuchen, ob Mikroplastiks toxischer ist als andere Partikel. Aufgrund dieser lückenhaften Datenlage ist auch weitestgehend unklar, welcher Faktor vornehmlich für die negativen Effekte von Mikroplastik verantwortlich ist: Die chemischen Inhaltsstoffe oder die partikulären Eigenschaften.

[23]Die Bambus-Mischung bildet eine Ausnahme. Allerdings wurde hier nur ein Produkt getestet.

Die vierte Studie dieser Arbeit (A4) trug zur weiteren Aufklärung der Mikroplastiktoxizität bei und betrachtete dazu Effekte auf den Wasserfloh *Daphnia magna*. Dabei rückten die Untersuchungen bisher vernachlässigte Faktoren in den Fokus, um die Heterogenität des in der Umwelt vorkommenden Mikroplastiks abzubilden. Ein erstes chronisches Experiment widmete sich weniger beachteten Polymeren. Hier wurden die Effekte von Mikroplastik aus PVC, PUR und PLA sowie dem natürlichen Partikel Kaolin, in Konzentrationen von 2, 10, 100 und 500 mg L^{-1}, auf Lebenszyklusparameter des Süßwasser-Invertebraten untersucht.[24] Alle drei Mikroplastiktypen hatten in dem getesteten Konzentrationsbereich negative Auswirkungen auf das Überleben, die Reproduktion und/oder das Wachstum von *D. magna*. Gleichzeitig waren diese Effekte stärker als die durch Kaolin hervorgerufenen Wirkungen. Die Exposition gegenüber PVC-Partikeln reduzierte die Reproduktion am stärksten, während die Exposition gegenüber PLA-Partikeln zur höchsten Mortalität führte. Dies weist darauf hin, dass jeder Mikroplastiktyp eine spezifische Toxizität besitzt. Ein zweiter Versuch sollte aufklären, ob die beobachteten Effekte durch die chemischen Inhaltsstoffe oder durch die physikalischen Eigenschaften des Mikroplastiks hervorgerufen wurden. Dazu wurden vier Expositionsszenarien miteinander verglichen: (1) Mikroplastik mit allen darin enthaltenen Chemikalien, (2) Mikroplastik ohne die mit Methanol extrahierbaren Substanzen, (3) Chemikalien, die sich mit Methanol aus dem Mikroplastik extrahieren lassen, d.h. im Kunststoff enthalten sind (4) Chemikalien, die sich mit Wasser aus den Partikeln lösen lassen, d.h. unter natürlichen Bedingungen freigesetzt werden. Im Fall von PVC beeinflussten ausschließlich die extrahierbaren Substanzen die Reproduktion und das Wachstum von *D. magna* während im Fall von PUR und PLA nur die Expositionen gegenüber Partikeln zu Effekten führten. Die Ergebnisse zeigen, dass die im Kunststoff enthaltenen Chemikalien für die negativen Auswirkungen von Mikroplastik verantwortlich sein können, dies allerdings vom Mikroplastiktyp abhängt. Entsprechend können auch die physikalischen Partikeleigenschaften die Toxizität des Materials bedingen.

Insgesamt macht die Studie deutlich, dass sich die Auswirkungen von Mikroplastik zu Mikroplastik unterscheiden. Für eine aussagekräftige ökotoxikologische Risikobewertung ist es daher essentiell die Diversität des in der Umwelt vorkommenden Materials stärker in der Forschung abzubilden. Laut den dargelegten Studienergebnissen sollten dabei insbesondere weniger betrachtete Polymere, wie PVC, PUR und der Biokunststoff PLA, sowie die

[24]Die getesteten Konzentrationen waren höher als die derzeit in der Umwelt gemessenen. Da es das Ziel war, den Mechanismus der Mikroplastiktoxizität zu untersuchen, wurden Konzentrationen gewählt, die sehr wahrscheinlich zu negativen Effekten führen.

heterogenen physikalischen und chemischen Charakteristika von Mikroplastik mehr Berücksichtigung finden.

Gleichzeitig stellt sich die Frage, ob sich die klassische toxikologische und ökotoxikologische Risikobewertung dafür eignet, das Risiko dieses komplexen, heterogenen und dynamischen Materials zu bestimmen. Hinzu kommt, dass die Auswirkungen von (Mikro)plastik nicht nur die Umwelt, sondern auch die Gesellschaft betreffen. Beispielsweise kann Plastikmüll am Strand zu Tourismuseinbußen führen. Die sozial-ökologische Risikoperspektive berücksichtigt neben der ökologischen auch die gesellschaftliche Komponente von Plastik. In dem Projekt PlastX, im Rahmen dessen die vorliegende Arbeit entstand, wurde diese Perspektive genutzt, mit dem Ziel die Rolle von Kunststoffen in der Gesellschaft und die damit einhergehenden Umweltauswirkungen zu untersuchen sowie Lösungsstrategien zu entwickeln. Um das anthropogene Phänomen Plastik in seiner Vielschichtigkeit zu beleuchten, wurden hierbei mittels eines inter- und transdisziplinären Forschungsansatzes verschiedene wissenschaftliche und gesellschaftliche Perspektiven integriert.

Die im Verlauf der vorliegenden Dissertation gewonnenen Erkenntnisse wurden mit denen aus der soziologisch-ethnographischen Forschung und der unternehmerischen Praxis in der fünften Studie dieser Arbeit integriert (A5). Gegenstand war die vielschichtige ‚Undurchschaubarkeit' der doch eigentlich ‚durchsichtigen' Plastikverpackung. Die Veröffentlichung verdeutlicht, dass es an Wissen mangelt (z.B. kennen selbst die Produkthersteller meist nicht alle Inhaltsstoffe ihres Produkts), dass vorhandenes Wissen verschwiegen wird (z.B. aufgrund von Konkurrenz oder mangelnder Ressourcen) oder dass verfügbares Wissen nicht anschlussfähig ist (z.B. sind wissenschaftliche Erkenntnisse aufgrund ihrer Komplexität schwer verständlich oder in der Praxis nicht umsetzbar). Dieser Mangel an Wissen und adäquater Kommunikation erschwert die Entwicklung und den Verkauf sicherer und nachhaltiger Verpackungen, da beispielsweise Produkthersteller und Handel diese ‚besseren' Verpackungen nicht identifizieren können. Basierend auf der herausgearbeiteten Problematik, leitet der zweite Teil der Publikation Handlungsempfehlungen für Politik, Wirtschaft und Wissenschaft ab, die eine kollaborative Gestaltung sicherer und nachhaltiger Verpackungen begünstigen.

Die vorliegende Arbeit untermauert zuvor geäußerte Zweifel an der toxikologischen Unbedenklichkeit von Kunststoffprodukten mit wissenschaftlichen Daten. Weitergehende Untersuchungen zur realistischen Gefahreneinschätzung sind essentiell und sollten beispielsweise die tatsächliche Exposition gegenüber Kunststoffchemikalien berücksichtigen.

Basierend auf den Ergebnissen der vorliegenden Arbeit, bringt ein Wechsel hin zu anderen Materialtypen, einschließlich ‘Biomaterialien‘, nicht automatisch eine Verbesserung mit sich. Allerdings gibt es bereits Produkte auf dem Markt, die keine toxischen Chemikalien enthalten. Diese Produkte können somit als Orientierung für die Entwicklung sicherer Kunststoffe und deren Alternativen dienen. Die produktspezifische chemische Zusammensetzung und die Vielzahl an unbekannten Substanzen in Kunststoffen machen eine Weiterentwicklung der derzeitigen Produkttestung zwingend notwendig, um die gesundheitliche Unbedenklichkeit von Kunststoffen zu garantieren. Die in dieser Arbeit angewandte Kombination aus In-vitro-Assays und chemischer Analytik zur Untersuchung der im Endprodukt enthaltenen bzw. daraus auslaugenden Chemikalienmischung stellt eine vielversprechende Alternative zur aktuellen Testweise dar. Durch die Charakterisierung der Mischtoxizität sowie der chemischen Zusammensetzung gleichermaßen, erlaubt die Methodik realistischere Einschätzungen der Toxizität eines Produkts als die ausschließliche Analyse absichtlich zugesetzter Einzelsubstanzen, wie sie derzeit praktiziert wird. Das Testverfahren ließe sich erleichtern, indem die Komplexität von Kunststoffprodukten reduziert würde. Beispielsweise könnte man nur eine limitierte Anzahl nicht schädlicher Polymere und Chemikalien in Form von Positivlisten zulassen. Gefördert durch Verordnungen und Richtlinien, ist gleichzeitig entlang der gesamten Lieferkette eine transparente Offenlegung all dieser in Kunststoffen enthaltenen Chemikalien anzustreben. Zudem sollte die Prüfung toxikologischer und ökotoxikologischer Effekte in Nachhaltigkeitsbewertungen von Materialien, wie Ökobilanzen, integriert werden, um ‚regrettable substitutions‘ vorzubeugen. Eine Kultur offener Kommunikation und Zusammenarbeit innerhalb und zwischen Wissenschaft und Wirtschaft bildet den essenziellen Rahmen, um Kunststoffe nachhaltig zu gestalten und zu nutzen.

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A9 Publications, conference contributions and media attention

A9.1 Publications

Peer reviewed journals

Zimmermann, L., Bartosova, Z., Braun, K., Oehlmann, J., Völker, C., Wagner, M. (*submitted*) Plastic products leach chemicals that induce *in vitro* toxicity under realistic use conditions.

Zimmermann, L., Dombrowski, A., Völker, C., Wagner, M. (2020) Are bioplastics and plant-based materials safer than conventional plastics? *In vitro* toxicity and chemical composition. Environment International 145, 106066. doi: 10.1016/ j.envint.2020.106066.

Zimmermann, L., Göttlich, S., Oehlmann, J., Wagner, M., Völker, C. (2020) What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*, Environmental Pollution 267, 115392. doi: 10.1016/j.envpol.2020.115392.

Zimmermann, L., Dierkes, G., Ternes, T. A., Völker, C., Wagner, M. (2019) Benchmarking the *in vitro* toxicity and chemical composition of plastic consumer products. Environmental Science & Technology 2019 53 (19), 11467–11477. doi: 10.1021/acs.est.9b02293.

Sattlegger, L., Haider, T., Völker, C., Kerber, H., Kramm, J., Zimmermann, L., Wurm, F. R. (2019) Die PET-Mineralwasserflasche. Wasser in Plastik, Plastik in Wasser. Chemie unserer Zeit 54 (1),14–20. doi: 10.1002/ciuz.201900875.

Markwart, J. C., Battig, A., Zimmermann, L., Wagner, M., Fischer, J., Schartel, B., Wurm, F. R. (2019) Systematically controlled decomposition mechanism in phosphorus flame retardants by precise molecular architecture: P–O vs P–N. ACS Applied Polymer Materials 2019 1 (5), 1118–1128. doi: 10.1021/acsapm.9b00129.

Steiner, K., Zimmermann, L., Hagenbuch, B., Dietrich, D. (2015) Zebrafish-Oatp mediated transport of Microcystin-congeners. Archieves of Toxicology. 2016 90 (5), 1129–1139. doi: 10.1007/s00204-015-1544-3.

Not peer reviewed journals

Kramm, J., Völker, C., Haider, T., Kerber, H., Sattlegger, L., Zimmermann, L. (2020) Sozial-ökologische Forschung zu Plastik in der Umwelt. Ergebnisse der Forschungsgruppe PlastX.

Sattlegger, L., Zimmermann L., Birnbach, M. (2020) Von der unsichtbaren zur durchschaubaren Verpackung. Prinzipien nachhaltiger Verpackungsgestaltung. Ökologisches Wirtschaften 35 (1), 38–42.

Zimmermann, L., Wagner, M., Völker, C. (2019) In-vitro-Toxizität und chemische Zusammensetzung von Kunststoffprodukten. GDCh Mitteilungen Umweltchemie und Ökotoxikologie, 25. Jahrg. 4/2019, 104–106.

A9.2 Conference contributions

Zimmermann, L., Völker, C., Wagner, M. (2020) Drivers of microplastic toxicity on *Daphnia magna*. MICRO2020, online conference, November 23–27, 2020 (platform presentation).

Zimmermann, L., Völker, C., Wagner, M. (2020) Recent analysis on the toxicities of bioplastics and plant-based materials. Food Packaging Forum, Zürich, Switzerland, October 21, 2020 (platform presentation).

Zimmermann, L., Völker, C., Wagner, M. (2020) *In vitro* toxicity and chemical composition of plastic consumer products – What is in and what is getting out? Society of Environmental Toxicology and Chemistry (SETAC) SciCon Europe 30th Annual Meeting, online meeting, May 03–07, 2020 (platform presentation).

Zimmermann, L., Völker, C., Wagner, M. (2019) Chemicals in plastics: Toxicity and Composition. Food Packaging Forum, Zürich, Switzerland, September 24, 2019 (webinar presentation).

Zimmermann, L., Völker, C., Wagner, M. (2019) *In vitro* toxicity of plastics: Are bioplastics indeed safer? SETAC German Language Branch (GLB) 24th Annual Meeting, Landau (Pfalz), Germany, September 04–06, 2019 (platform presentation).

Zimmermann, L., Völker, C., Wagner, M. (2019) Are "bioplastics" a safe alternative to conventional plastics? *In vitro* toxicity of extracts from plastic products. SETAC Europe 29th Annual Meeting, Helsinki, Finland, May 26–30, 2019 (platform presentation).

Zimmermann, L., Völker, C., Wagner, M. (2018) Have your say: What are the risks of microplastics? MICRO2018, Lanzarote, Spain, November 19–23, 2018 (poster presentation).

Zimmermann, L., Völker, C., Wagner, M. (2018) What is in our plastic? *In vitro* toxicity of extracts from plastic products. SETAC Europe 28th Annual Meeting, Rome, Italy, May 13–17, 2018 (platform presentation).

Zimmermann, L., Völker, C., Wagner, M. (2018) How toxic are our daily used plastic products? Screening the toxicity of plastic extracts with *in vitro* bioassays. 7th Young Environmental Scientists Meeting, Madison, Wisconsin, March 25–29, 2018 (platform presentation).

Zimmermann, L., Völker, C., Wagner, M. (2017) In vitro-Toxizität von Kunststoffextrakten. SETAC GLB 22th Annual Meeting, Neustadt an der Weinstraße , Germany, November 12–14, 2017 (platform presentation).

Zimmermann, L., Völker, C., Wagner, M. (2017) *In vitro* analysis of leachates from plastic packaging – Study design. 6th Young Environmental Scientists Meeting, University Stockholm, Sweden, February 16–20, 2017 (poster presentation).

Kramm J., Völker, C., Haider, T. Kerber, H. Sattlegger, L. Zimmermann, L. (2017) How to cope with plastic waste in the environment? Finding solutions through a transdisciplinary research approach. ASLO 2017 Aquatic Sciences Meeting, Mountains to the Sea, Honolulu, Hawai`i, February 26 – March 03, 2017 (platform presentation).

Zimmermann, L., Völker, C., Wagner, M. (2016) Risk assessment of microplastics in freshwaters. 7th Late Summer Workshop „Microplastics in the Aquatic Environment“, Haltern am See, Germany, September 25–28, 2016 (poster presentation).

A9.3 Media attention – A selection of the different media

Television

Hr alles wissen *Giftstoffe im Plastik*. September 24, 2020

Hr Die Ratgeber *Gefahren die in Kunststoff stecken*. March 02, 2020

Hr Hessenschau *Untersuchungen zu Plastikverpackungen*. September 30, 2019

Radio

ORF 1 Help – Das Konsumentenmagazin *Einwegverpackungen könnten giftiger sein als gedacht*. January 30, 2021

Deutschlandfunk *Bioplastik – Toxikologisch kein Unterschied zu konventionellen Kunststoffen*. November 20, 2020

BR 2 IQ Science and Research *Plastics in everyday life – How dangerous is the chemical cocktail in plastics?* October 16, 2019

Podcasts

The Zero Waste Countdown Podcast with host Laura Nash *102. Toxins In Bioplastic*. October 25, 2020

The Zero Waste Countdown Podcast with host Laura Nash *102. Toxins In Our Plastic Products*. August 02, 2020

Plastisphere Podcast by Anja Krieger *Plastisphere – Ep.7: Confused about bioplastics?* July, 2019

Print and online articles

Science for Environment Policy, news service published by the European Commission's Environment Directorate-General *Researchers use advanced techniques to characterise chemicals found in everyday plastics*. Issue 546, July 21, 2020

Chemical watch *Plastics cause* in vitro *toxicity via unknown mixtures, study finds*. September 19, 2019

Spektrum *Researchers find over 1100 unknown ingredients.* September 17, 2019